

REPORT OF A CASE OF TRUE LATERAL HERMAPHRODITISM IN SUS

PAUL E. NIELSON

Department of Anatomy, University of Wisconsin, Madison

TWO PLATES (NINE FIGURES)

True lateral hermaphroditism in mammals is still enough of a rarity to warrant description of a pig which was discovered in routine inspection at a local packing plant in March, 1940. The specimen was brought to our attention through the courtesy of Dr. D. H. Nelson, bacteriologist at the plant. Dr. S. C. Varnum, of the Bureau of Animal Industry, recognized the nature of the tract and saved it for study.

Pseudohermaphroditism occasionally occurs in the pig, but true hermaphroditism is quite rare. In the latter case the gonads may be a single ovary on one side and testis on the other side, or two of each, or an ovary paired with an ovotestis, a testis paired with an ovotestis, or two ovotestes. The lateral type, having a separate testis on one side and ovary on the other side, and no ovotestis tissue, is the rarest of all combinations. True lateral hermaphrodite pigs of this type have been described by Kingsbury ('09), Ancel ('20) and Corner ('21 b). Gould ('23) was able to study such a pig shortly before it was slaughtered and was also able to give a detailed anatomical description after it was killed. Crew ('24), while giving no data on the numbers of pigs having the various combinations, has an illustration of true lateral type, and he also writes of a pig having paired ovaries within the abdominal cavity and paired testes underneath the skin of the perineum. In all of these true lateral hermaphrodites the ovary was found on the left side and the testis on the right. The

most recent study from a physiological standpoint was one by Zuckerman and Groome ('40) on a pseudohermaphrodite pig with scrotal testes which were histologically of the cryptorchid type. This latter paper and the chapters by Willier and Witschi in *Sex and Internal Secretions* (Allen, et al., '39) contain the best discussions and summaries of the literature available on the general subject.

On gross examination the specimen to be described in this paper had an ovary on the left side and a testis on the right (fig. 1). The ovary was partially surrounded by the fimbriated end of the oviduct, and the latter was continued down to the left uterine horn. On the right side the oviduct ended blindly after passing along the dorsolateral border of the testis for about two-thirds of the length of that organ.

The uterine horns were quite large and evenly distended in the fresh state, as in an early stage of pregnancy. They possessed marked longitudinal ridges after fixation as is so often seen in the multiparous uterus. The upper end of the right uterine horn, as it approached the testis, was somewhat smaller in diameter than the left horn. On opening the fixed uterus no embryos were found following careful inspection. The uterine lumen was irregularly dilated and in the larger dilated areas the mucosa was very smooth with large leaf-like folds of the mucosa lying closely pressed against one another, somewhat shingle-like in appearance. In the constricted portions the lumen was very irregular with high folds of the mucosa projecting into it. The specimen included the upper cervix, but no vagina was available for study.

The ovary was about one and one-half times as large as the normal ovary of the sow, and contained several corpora lutea and medium-sized follicles. Some of the corpora lutea were cystic, but this is not unusual for the normal ovary.

The male organs present were the testis, epididymis and vas deferens, and these were all present on the right side only. Grossly, the testis was smooth in appearance and somewhat brownish in color. Capping over its cephalic pole was a lobe of the epididymis and this was partially separated from a

more posterior lobe of the same structure by a shallow incisure. The epididymis was continued out onto the broad ligament as the vas deferens which was quite coiled at first but soon became less tortuous and larger in diameter as it approached the cervix of the uterus. Gross dissection showed that it did not open into the uterine horn or upper cervix, but it may have opened lower down in the portion of the tract not available for study.

On gross examination the ovary appeared to be just as in the normal animal. The testis, although abdominal in position, did not appear significantly different than the testis of the normal boar, except that it was possibly slightly darker in color. Histological sections of large portions of each gonad failed to reveal any ovotestis tissue in either of them, hence serial sections were not made. Microscopically the ovary was found to contain some corpora albicantia and some normal active corpora lutea (fig. 3), which appeared to fit Corner's description of about 10 days post-ovulation (Corner, '21 a). Follicles in every stage up to a well developed medium-sized one were present. Some of them were atretic while others appeared normal. The theca interna around the larger follicles was about four to five cells thick. There were quite a few eosinophile leukocytes in the ovarian stroma, and especially near the atretic follicles.

The epithelium of the oviduct was high, tending toward stratification of the nuclei (fig. 9). The mucosa of the tube in the lower region of the ampulla was folded and in the crypts of the folds the epithelium was evaginated into small pouches, somewhat glandular in appearance. These small pouches contained much mucinous material in strands in their lumina, but the lumen of the oviduct was free from staining material.

The uterus was lined by a tall columnar epithelium which clothed the numerous leaf-like folds of the mucosa (fig. 7). The glands had a high secretory type of epithelium with a granular cytoplasm, a clear basal zone in many of the glands, and secretion products in the lumen and on the surface of the cells (fig. 8). The glands did not appear quite as numer-

ous as in the normal pig of a comparable stage, although the larger lumen may account for much of this apparent difference. As in the normal pig uterus, the uterine glands of the hermaphrodite invaded the musculature for a short distance. The mucosal connective tissue was free from eosinophile leukocytes, but there were quite a few lymphocytes, none of these were in the epithelium, however. Nothing unusual was noted in the cervix. No hyaline degeneration was noted in the walls of any blood vessels.

The testis was the typical cryptorchid type with what appeared to be a hypertrophy and hyperplasia of the interstitial tissue and with no spermatogenic tissue (figs. 4 and 5). Each of the seminiferous tubules contained only Sertoli cells which filled most of each lumen with their syncytial processes. The interstitial cells appeared active, possessing many granules in their cytoplasm. The tissue was very vascular. The testis was not serially sectioned in an effort to establish whether or not the vasa efferentia connected the tubules to the epididymis. The epididymis had a tall pseudostratified ciliated epithelium (fig. 2), not quite as tall, however, as seen in some normal males examined. Some tunnels in the epithelium of this organ were noted. The section of the vas deferens near the cervix of the uterus possessed a tall columnar epithelium (fig. 6). No seminal vesicles were present on the tissue available although Gould ('23) noted them near the cervix in the specimen he examined. Possibly they were present lower down in this instance.

The histological picture of the testis is much like that reported by Zuckerman and Groome for a pseudohermaphrodite pig which they studied. They established the normal histological picture for their animal by biopsy of the several genital organs and then began injections of testosterone propionate, continuing these injections for $2\frac{1}{2}$ months shortly after which tissue was again removed for study. The animal was then injected with estradiol benzoate for a similar period of time and portions of the sexual organs again removed for study. The pig was then castrated. Comparison of the histological

changes taking place in these organs under the several procedures led Zuckerman and Groome to conclude that the testes of their pseudohermaphrodite were secreting predominately estrogenic hormone or this even to the complete exclusion of any androgenic hormone. The site of production of this hormone was postulated by these authors as being the interstitial cells and possibly even the Sertoli cells, although it was thought improbable that these latter cells were the site. In our own specimen the histological picture of the uterus suggests an early luteal phase of stimulation. This is in keeping with the presence of an active corpus luteum in the ovary. The histological picture of the interstitial cells of the testis shows them to be very active with many of the cells possessing a peripheral vacuolated zone, giving these cells an appearance much like luteal cells. The well preserved epithelium of the male accessory organs shows that some hormone is present capable of keeping up the epithelium of these organs. The possibility is present, therefore, that this animal had an ovary and a testis which were both actively secreting, although they were possibly not equally effective. It does not necessarily follow that the testis in our specimen functioned the same as in the case reported by Zuckerman and Groome, nor can it be definitely said that the testicular tissue did not possess the same function in the two animals. The presence of an apparently active corpus luteum in our specimen complicates the interpretations of the histological data.

SUMMARY

This is a case of true lateral hermaphroditism in which the ovary lies on the left side and the testis on the right. Grossly, the ovary resembled in every respect that of the normal sow. The testis was abdominal and histologically of the cryptorchid type. Histological examination of considerable portions of each gland failed to reveal any mixture of ovarian and testicular tissues in either of them. Serial sections were not made but there was no indication that either organ could have

been an ovotestis. In all other true lateral hermaphrodites of the pig hitherto described the ovary was also on the left side and the testis on the right. The corpora lutea and uterus indicate that this animal had probably ovulated about 10 days before being killed. The height of the epithelium in the oviducts, uterus, epididymis and vas deferens indicates adequate hormonal stimulation. The presence of this well preserved epithelium in these organs suggests possible secretory activity of both the testis and the ovary. The active corpus luteum no doubt is the source of the ovarian secretion, and the interstitial cells of the testis are the most probable site of the secretion from that gonad although the possibility exists that the Sertoli cells may contribute. This latter possibility is not very likely and probably not necessary to postulate in this instance. The lack of experimental evidence from this animal and other similar cases makes it impossible to adequately explain the development and physiology of these interesting anomalies. This communication is of value then in adding another case to the, as yet, limited number of true hermaphrodites known to occur among mammalian species.

I am indebted to Drs. H. W. Mossman and R. K. Meyer for valuable criticisms and suggestions made in the course of the study.

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PLATE 1

EXPLANATION OF FIGURES

1 Internal reproductive tract of a hermaphrodite pig, as seen from the ventral side. The ovary can be seen in the upper portion of the figure, partially covered (on the right in the figure) by the fimbriated end of the oviduct. Visible as swellings on the ovary are several corpora lutea, the uppermost one is cystic. In the lower half of the figure the male portion of the intersex is visible. Beginning near the cervix of the uterus, lying in the broad ligament, the vas deferens can be seen as a small tubular structure. The overlying serosa of the mesometrium has been removed to make the vas more easily seen. A small portion of the serosa was removed from the mesometrium on the ovarian side to show that no vas deferens was present here. The vas can be followed up to the enlarged epididymis (cauda); the caput epididymis was removed for histological study and the location it previously occupied is outlined. The testis lies in the pocket made by the epididymis and right uterine horn; the area removed for microscopical study has been outlined. Between the testis and the cauda epididymis the terminal end of the small right oviduct can be seen. Approximately $\times \frac{1}{2}$.

2 Section through the epididymis showing the high ciliated pseudostratified epithelium lining the tubules of that organ. $\times 95$.

3 Section from near the periphery of a corpus luteum. The smaller cells at the left are possibly theca interna cells which appear to be invading the tissue which developed from the granulosa. $\times 180$.

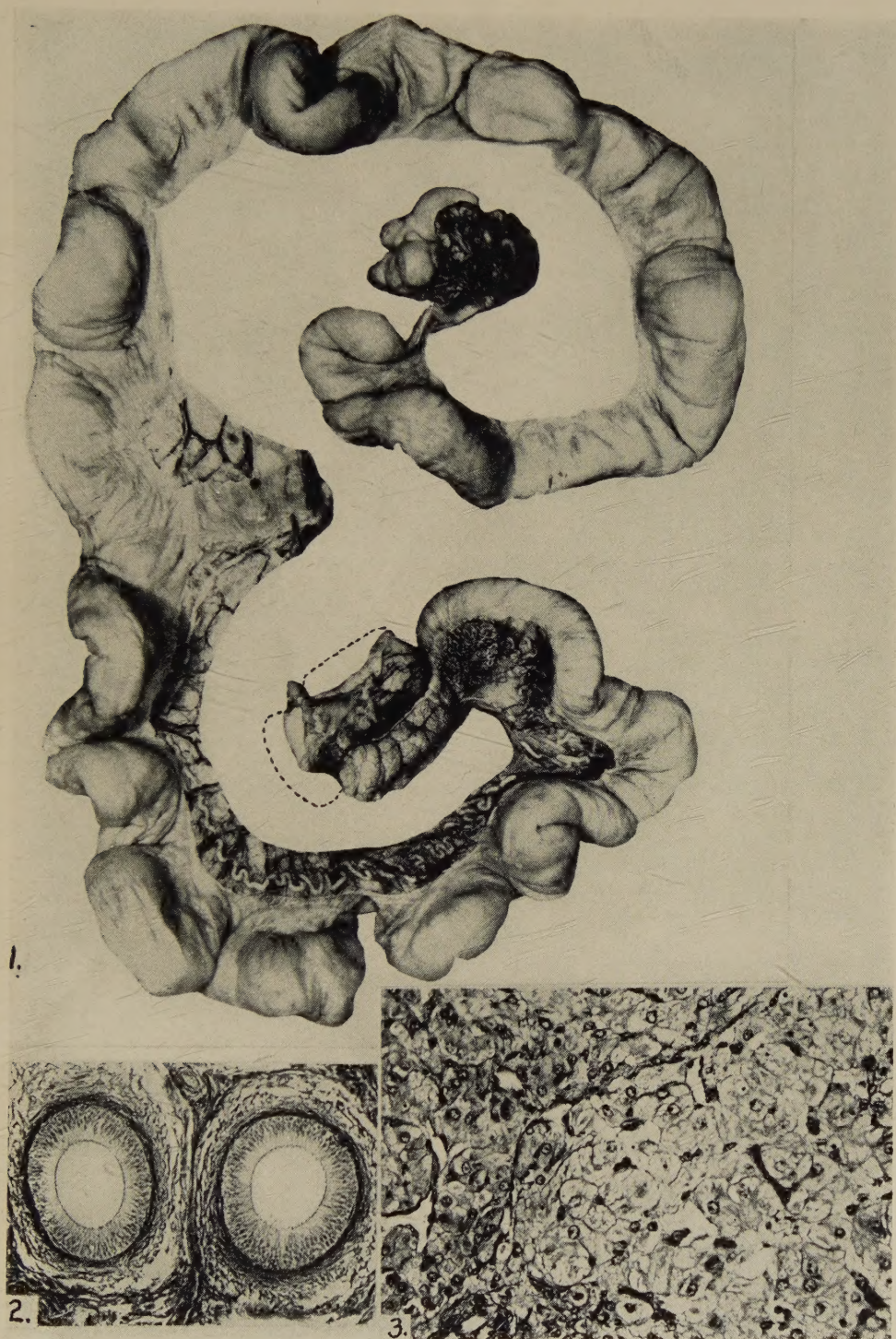


PLATE 2

EXPLANATION OF FIGURES

4 Section of cryptorchid testis as seen under low power, showing the great amount of interstitial tissue between the seminiferous tubules. These tubules are free from spermatogenic cells, only the Sertoli cells remain. $\times 90$.

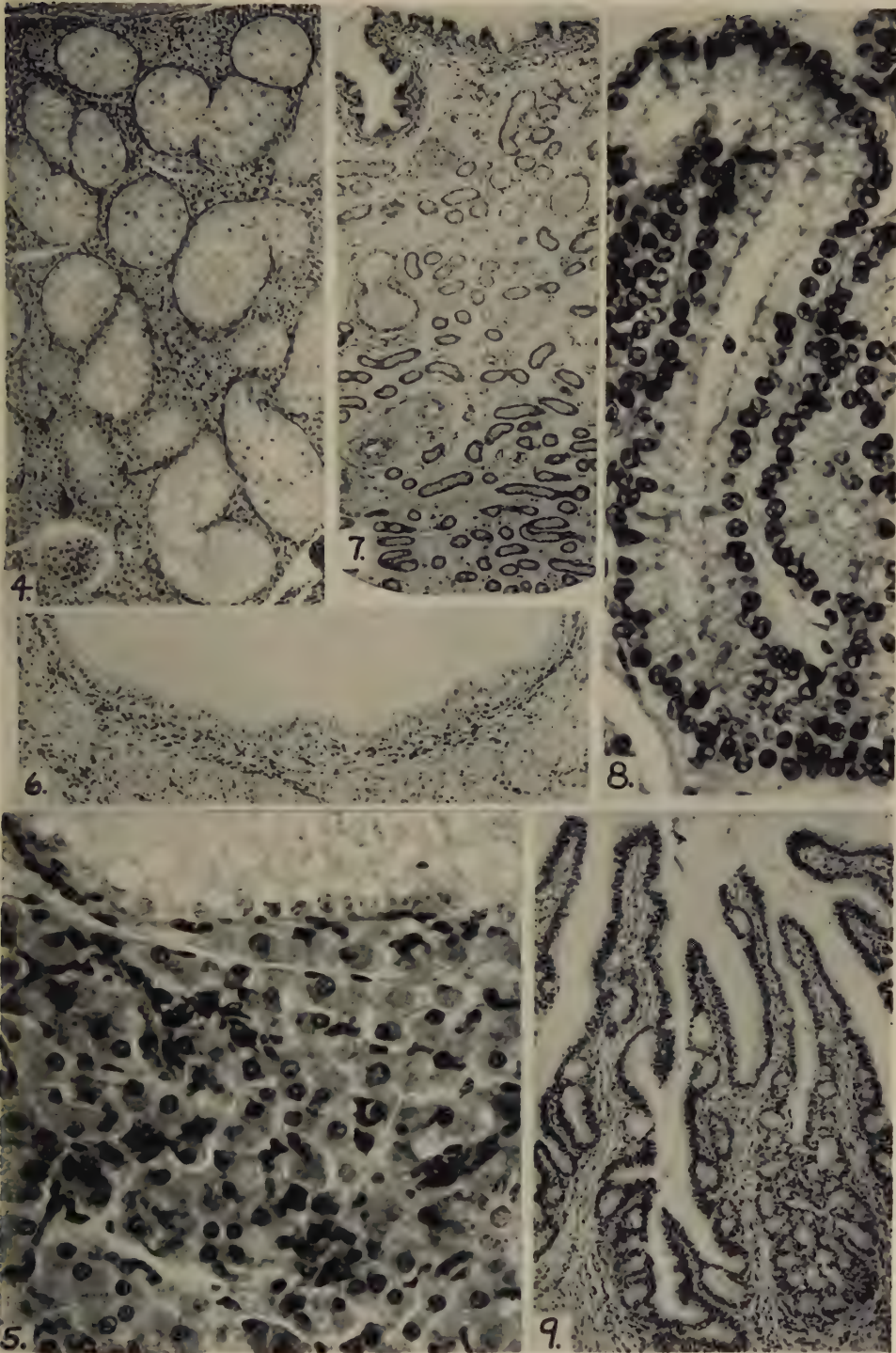
5 Section of testis under higher magnification than in figure 4, showing interstitial cells with their peripheral clear border of lipoid material. A portion of a seminiferous tubule is shown near the upper margin. $\times 430$.

6 Section of the vas deferens taken from near the cervix of the uterus. The tall columnar epithelium is apparent. $\times 90$.

7 Low magnification of a section of the mucosa of the uterus showing the high epithelium lining the lumen (above) of the uterus. The actively secreting glands are somewhat dilated near the lumen of the uterus, but deeper in the mucosa the glands are smaller and have a reduced lumen. $\times 55$.

8 Section of uterine gland under higher magnification than in figure 7. This gland was near the free surface of the mucosa and shows secretion droplets lying in the lumen on the surface of the cells, and also shows the narrow clear basal zone which is present in some portions of the gland epithelium. $\times 470$.

9 Section low in the ampulla of the oviduct showing the high epithelium over all the tissue and prominent gland-like outpouchings in the crypts. $\times 95$.



SEX DIFFERENCE IN GROWTH IN GONAD-ECTOMISED ALBINO RATS

Y. Z. TANG¹

Institute of Animal Genetics, University of Edinburgh, Scotland

THREE FIGURES

A review of literature on the effect of gonadectomy on growth in rats shows some controversy among investigators, especially the effect on body weight of castrated rats following castration. The present study aimed not only to reinvestigate the problem on body growth as a whole, but all the internal organs were studied individually along with food consumption. Furthermore, it seemed interesting to make comparisons between male and female rats after gonadectomy in order to see to what extent sex difference in growth and food utilization would change as a result of the removal of gonads.

The author wishes to express his thanks and gratitude to Prof. F. A. E. Crew, F.R.S., for his generous hospitality which made this study possible. He also gratefully acknowledges the constant interest and assistance of Dr. H. P. Donald during the entire experiment period. Lastly, he is indebted to Dr. L. M. Winters of Minnesota for reading the manuscript and giving helpful criticisms.

MATERIAL AND METHODS

The animals used in this experiment were selected from the Wistar strain albino rats of this laboratory. A total of seventy-five rats were used, comprising thirty-four males and forty-one females. They were from ten litters. Approximately half of the animals of each sex in each litter were used as controls for the other half on which gonadectomy was performed.

¹ Recently appointed staff member, Department of Animal Husbandry and Veterinary Science, National Central University, Chengtu, Szechuen, China.

The rats have been subdivided into two other major groups according to the age at which they were gonadectomised. Group A consists of those which were gonadectomised shortly after birth together with their controls. It includes six litters, with forty-two rats, two litters were operated on the day of birth, and four at 2 weeks of age. Group B including thirty-three rats from four litters, were gonadectomised at 45 days of age. Incisions were made on controls and their gonads were examined but kept intact. Incidentally it has been observed that among the rats used in this experiment the time of the opening of vagina in female rats was about 41 days of age.

All the animals were housed in individual cages and fed ad libitum with a synthetic diet ensuring satisfactory growth and maintenance of health. The measured feeding period lasted for 10 weeks in each group. For group A animals this period began at 30 days of age, and for group B at 48 days.

At the end of the experimental period all the animals were killed and completely autopsied, and the weights of organs taken. The skeletons were macerated for later measurement.

OBSERVATIONS AND DISCUSSION

Growth rate

The gain in body weight in grams during the 10 weeks of experimental period for different groups is summarized as follows:

TABLE 1

Average body weight increase of rats in 10 weeks (number of rats in brackets)

	MALE RATS		FEMALE RATS	
	Group A	Group B	Group A	Group B
Controls	169.1(9)	134.8(9)	113.2(12)	87.3(9)
Gonadectomised	151.0(8)	123.5(8)	130.0(13)	109.0(7)
Difference	-18.1	-11.3	+16.8	+21.7

It is evident from this table that castration of male rats has reduced the gain in weight as compared with the control males irrespective of the time of castration. While in females

the spayed animals are heavier in both groups than their controls. Statistical treatment of these data revealed that the effects of gonadectomy on body weight increase were significant.

Comparing the males with females, it will be seen that both castrated and control males exceeded in growth rate the spayed and control females. It is of interest to compare the gonadectomised animals. The removal of the gonads should tend to eliminate sex difference insofar as these depend on the functioning of the gonads. The removal of the testes retards weight increase while removal of ovaries accelerates it so that gonadectomy causes the growth curves to become an intermediate type between those for normal males and females. Reference to figure 1 will show how the sex difference, although reduced by gonadectomy, is not completely eliminated. The remaining difference is still in favor of the males.

As judged by the main increase in weight, early castration and spaying has been no more effective in reducing the sex difference than the later. The difference between the control males and females has been reduced by about five-eighths by early gonadectomy, and by a little under six-eighths by the later. Those animals which were operated upon on the day of their birth showed no apparent difference from the rest of the group. Since gonadectomy can only be performed after birth, characteristic sex difference has already developed to a certain extent. Though it may be minimized by preventing further development of the reproductive organs, it can not be entirely eliminated.

The size of an animal is predetermined by certain genetic factors, and it is later subject to many environmental factors. Nutrition, temperature, light, etc., all affect the ultimate size, but the endocrine organs play a very important part in growth. A male and a female may be genotypically homozygous so far as size determining factors are concerned, yet their growth is conditioned by sex. Since the female is handicapped

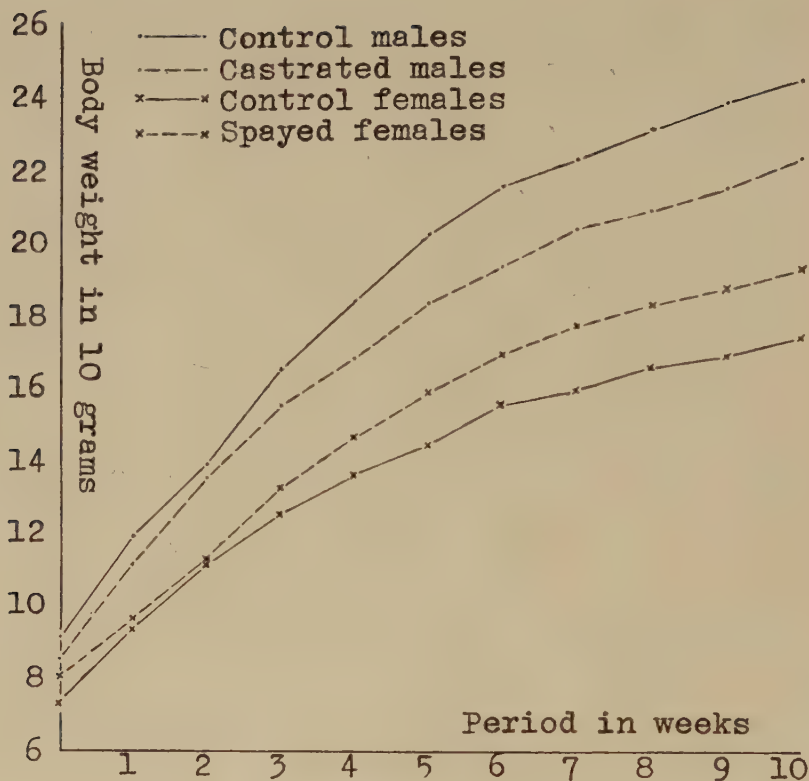


Fig. 1 Growth curves of rats.

in its growth by the presence of ovaries, it still possesses the hereditary capacity to growth as soon as the ovaries are removed.

Growth response can also be evoked after retardation by other methods. Body weight of rats was held by Jackson ('36) at nearly constant weight (about 50 gm.) for 15 weeks by a protein-deficient diet. When refed on normal stock diet, the female rats had overtaken at 9 months of age the controls in average body weight. Similar results were observed in another experiment (Jackson, '37) on underfeeding. These examples of compensatory growth can be interpreted as due

to the inherited character of size. Modifications may be made by internal or external environmental factors, but whenever there is a chance to escape from such influence animals tend to show some form of compensatory growth.

Food consumption

The amount of feed consumed by each animal during the 10 weeks is given below (in grams) :

TABLE 2

Average food consumption during 10 weeks (number of animals in brackets)

	MALE RATS		FEMALE RATS	
	Group A	Group B	Group A	Group B
Controls	1189(9)	1160(9)	1042(12)	1038(9)
Gonadectomised	1089(8)	1068(8)	1031(13)	971(7)
Difference	—100	—92	—11	—67

It will be observed that in all cases the control rats ate more than gonadectomised. The control males exceeded the castrated males in both body weight and food consumption. The food consumption of control females, however, is higher than that of the spayed in spite of the fact that they put on less weight.

Efficiency quotient

The calculation of the efficiency quotients was made according to the formula of Winters and McMahon ('33) :

$$\text{E.Q.} = \frac{\text{gain in weight (grams)}}{\text{food consumed (grams)}} \cdot \frac{\text{initial weight} + \frac{1}{2} \text{ gain}}{100}$$

The object of the calculations was to arrive at an estimation of efficiency of food utilization which would allow for the varying live weight. The results of calculations are summarized as follows :

TABLE 3
Average efficiency quotients of experimental rats

WEEK	MALE RATS			FEMALE RATS			CONTROLS ♂ — ♀	GONAD- ECTOMISED ♂ — ♀
	Controls	Castrated	C-G	Controls	Spayed	C-G		
		%	%			%	%	%
1	0.344	0.282	18	0.220	0.181	18	36	36
2	0.254	0.305	—20	0.211	0.209	—4	21	31
3	0.335	0.255	24	0.166	0.228	—39	51	11
4	0.256	0.184	28	0.124	0.199	—60	52	—8
5	0.281	0.225	20	0.109	0.180	—66	61	20
6	0.213	0.179	16	0.139	0.155	—11	35	13
7	0.132	0.157	—18	0.078	0.123	—58	41	21
8	0.122	0.086	30	0.077	0.091	—12	37	—5
9	0.143	0.121	16	0.056	0.079	—43	61	34
10	0.118	0.169	—43	0.077	0.100	—30	35	41
Mean	0.220	0.196	7	0.125	0.155	—31	43	19

C, control; G, gonadectomised.

Consideration of the above table shows that the differences week by week between the castrated and control males are not consistent in sign nor in magnitude. For the females, however, the difference in E.Q. between the spayed and controls is in the same direction in the bulk of the paired values. Some confidence may be felt that spaying has resulted in a greater efficiency in utilization of food. For easy appreciation of the relative size of difference in efficiency, the differences are expressed in per cent in columns 4, 7, 8 and 9 in the preceding table. It shows that the average weekly superiority of the male controls over the female controls was 43%. Castration and spaying reduced this sex difference to 19%. Similarly, control males were 7% more efficient than the castrated, and the control females 31% less efficient than the spayed. The regularity with which the males have a higher E.Q. than the corresponding females leaves no doubt that they are more efficient either as normal or as castrated males.

There is a tendency apparent in both sexes for the rats to become less efficient as they grow older and their rate of growth diminishes. This is to be expected since their maintenance requirements remained comparatively constant while the use of food for growth decreased with the advance of age.

TABLE 4
Comparison of carcass measurements of castrated and control rats. Weights in grams and lengths in centimeters

ORGAN OR PART	18 CONTROL RATS				16 CASTRATED RATS				DIFFERENCE		F	SIGNIFI- CANCE
	Actual	Mean Relative	C.V.	Actual	Mean Relative	C.V.	Actual	Per cent of control				
Total body weight	235.2	100	7.4	214.6	100	9.6	-20.6	-8.8	10.0	SS		
Nose-anus length	22.26	9.46	4.4	21.69	10.11	4.9	-0.57	-2.6	2.6	NS		
Tail length	18.18	7.73	7.3	17.91	8.35	7.0	-0.27	-1.5	<1	NS		
Head weight	19.19	8.16	9.2	18.09	8.43	7.3	-1.10	-5.7	<1	NS		
Skeleton, muscle and spinal cord	132.2	56.19	9.3	124.4	57.97	11.2	-7.8	-5.9	3.0	NS		
Integument	31.74	13.49	12.3	29.93	13.95	15.7	-1.81	-5.6	1.5	NS		
Fat	8.54	3.63	45.5	8.91	4.15	48.6	+0.37	+4.2	<1	NS		
Brain weight	1.688	0.717	7.9	1.659	0.773	6.8	-0.029	-1.7	<1	NS		
Hypophysis weight	0.011	0.0047	21.1	0.014	0.0066	15.9	+0.003	+26.6	13.8	SS		
Eyeballs	0.207	0.088	9.7	0.202	0.094	13.9	-0.005	-2.5	<1	NS		
Submaxillary gland	0.503	0.214	11.2	0.473	0.220	14.7	-0.030	-5.9	1.9	NS		
Thyroid gland	0.018	0.0075	18.7	0.018	0.0083	23.9	+0.000	+1.8	<1	NS		
Thymus gland	0.218	0.093	26.3	0.318	0.148	15.2	+0.101	+46.3	30.2	SS		
Heart weight	1.199	0.510	14.4	1.145	0.534	16.7	-0.054	-4.5	<1	NS		
Lungs weight	2.146	0.91	28.7	2.255	1.051	24.6	+0.109	+5.1	<1	NS		
Liver weight	12.39	5.27	9.6	10.36	4.83	11.8	-2.03	-16.6	24.1	SS		
Spleen weight	0.839	0.356	24.8	0.882	0.411	27.5	+0.043	+5.1	<1	NS		
Suprarenal weight	0.042	0.018	12.6	0.049	0.023	21.3	+0.007	+16.6	6.3	S		
Kidneys weight	1.117	0.475	10.9	1.769	0.824	11.6	+0.652	+18.6	27.6	SS		
Stomach and contents	3.289	1.398	43.1	2.567	1.196	44.8	-0.722	-22.0	2.6	NS		
Stomach, empty	1.117	0.480	11.5	1.190	0.550	18.2	+0.073	+6.5	1.5	NS		
Intestines with contents	18.02	7.66	14.6	17.01	7.93	13.9	-1.01	-5.6	1.4	NS		
Intestines, empty	5.75	2.44	27.3	5.45	2.54	19.1	-0.30	-5.3	2.2	NS		
Testes weight	2.073	0.881	9.5									
Epididymes weight	0.871	0.370	30.2									
Other male accessories	1.564	0.665	36.7	0.069	0.032	63.6	-1.495	-95.6	101	SS		

C.V., coefficient of variability in per cent. The 5% and 1% points of F are 4.15 and 7.5 respectively.

TABLE 5
Comparison of carcass measurements of spayed and control females. Weights in grams and lengths in centimeters

ORGAN OR PART	21 CONTROL RATS			20 SPAYED RATS			DIFFERENCE		F	SIGNIFI- CANCE
	Actual	Mean Relative	C.V.	Actual	Mean Relative	C.V.	Actual	Per cent of control		
Total body weight	165.6	100	8.9	185.5	100	7.9	+19.9	+12.0	18.6	SS
Nose-anus length	20.24	12.23	4.3	20.71	11.16	3.3	+0.47	+2.3	3.5	NS
Tail length	16.54	9.99	7.8	17.70	9.54	7.1	+1.16	+7.0	8.5	SS
Head weight	14.86	8.98	6.3	16.48	8.88	8.6	+1.62	+10.9	18.6	SS
Skeleton, muscle and spinal cord	90.00	54.36	10.7	104.20	56.16	9.1	+14.2	+15.8	22.7	SS
Integument	21.37	12.91	14.1	25.12	13.54	14.1	+3.75	+17.6	13.3	SS
Fat	8.16	4.93	57.4	10.03	5.41	38.1	+1.87	+22.5	1.9	NS
Brain weight	1.566	0.946	6.2	1.618	0.870	7.5	+0.052	+3.3	2.3	NS
Hypophysis weight	0.0118	0.007	19.0	0.0126	0.007	17.8	+0.0008	+7.1	1.5	NS
Eyeballs	0.163	0.098	18.1	0.174	0.094	16.8	+0.011	+7.2	1.6	NS
Submaxillary gland	0.390	0.236	15.3	0.416	0.224	14.1	+0.026	+6.6	1.9	NS
Thyroid gland	0.0161	0.010	14.1	0.0158	0.009	12.7	-0.0003	-1.9	<1	NS
Thymus gland	0.2262	0.137	25.1	0.2969	0.160	22.5	+0.0707	+31.3	13.3	SS
Heart weight	0.815	0.492	15.5	0.948	0.511	13.8	+0.133	+11.4	5.6	S
Lungs weight	1.785	1.080	20.6	1.954	1.052	17.4	+0.169	+9.5	2.3	NS
Liver weight	8.81	5.32	10.9	9.22	4.97	11.3	+0.41	+4.6	1.6	NS
Spleen weight	0.711	0.429	28.7	0.721	0.389	17.3	+0.010	+1.4	<1	NS
Suprarenal weight	0.066	0.040	16.1	0.059	0.032	18.5	-0.007	-11.5	4.9	S
Kidneys weight	1.615	0.975	9.7	1.665	0.897	8.7	+0.050	+3.1	1.1	NS
Stomach and contents	2.603	1.572	42.2	2.871	1.547	43.0	+0.268	+10.3	1.8	NS
Stomach, empty	0.928	0.561	11.7	1.071	0.577	20.6	+0.143	+15.4	7.0	S
Intestines with contents	16.27	9.83	10.7	17.14	9.24	11.8	+0.87	+5.4	2.2	NS
Intestines, empty	4.52	2.73	24.9	4.80	2.59	26.0	+0.28	+6.2	1.8	NS
Ovaries	0.074	0.045	28.1							
Uterus	0.469	0.283	37.1	0.066	0.036	67.7	-0.403	-85.9	101	SS

C.V., coefficient of variability in per cent. The 5% and 1% points of F are 4.09 and 7.33 respectively.

TABLE 6

The significance of differences between means for males and females expressed as percentages of means for males

ORGAN OR PART	CONTROLS		GONAECTOMISED	
	Per cent difference $\sigma \sigma - \text{♀♀}$	Significance	Per cent difference $\sigma \sigma - \text{♀♀}$	Significance
Total body weight	29.61	SS	13.60	SS
Nose-anus length	9.07	SS	4.52	SS
Tail length	9.02	SS	1.17	SS
Head weight	22.56	SS	8.90	SS
Skeleton, muscle and spinal cord	46.94	SS	16.22	SS
Integument	32.67	SS	16.07	SS
Fat	4.19	NS	—12.62	NS
Brain weight	7.23	SS	2.47	NS
Hypophysis weight	—5.85	NS	10.38	NS
Eyeballs	21.56	SS	13.76	SS
Submaxillary gland	22.42	SS	12.05	S
Thyroid gland	9.60	NS	11.91	NS
Thymus gland	6.75	NS	—3.90	NS
Heart weight	28.97	SS	17.14	SS
Lungs weight	13.35	NS	16.82	S
Liver weight	11.10	SS	28.88	SS
Spleen weight	15.19	NS	18.21	S
Suprarenal glands	—19.45	S	—57.37	SS
Kidneys	25.68	SS	5.88	NS
Stomach and contents	—11.84	NS	20.86	NS
Stomach, empty	16.92	SS	—10.00	NS
Intestines and contents	9.71	S	—0.76	NS
Intestines, empty	21.41	SS	11.92	NS

SS, significant at the 1% point; S, significant at the 5% point; NS, non-significant. Weights in grams and lengths in centimeters.

Growth of the body

Body weight. Castrated male rats in both groups were found to be lighter than their controls, the difference being significant at the 1% point. The same degree of certainty attached to the differences found between the test and control females, between the male and female controls, and male and female test rats. These results are in agreement with the findings of Evans and Simpson ('27), van Wagenen ('28), Korenchevsky ('34) and of Lawless ('36). But Slonaker ('30)

observed greater body weight in castrated male rats, while Stotsenburg ('09), Hatai ('13, '15) working with albino rats and Masui and Tamura ('26) in mice reported that castration did not materially make any difference so far as the body weight was concerned. Holt, Keeton and Vennesland ('36) found overlapping results in their experiment and considered it inconclusive.

That female rats grow to a heavier body weight after ovariectomy is almost universally recognized. Freudenberger and Clausen ('37) postulated that oestrin had the inhibitive effect on body weight growth.

Nose-anus length (body length). As it is pointed out by van Wagenen ('28) that body length shows less response to castration than tail length, it is therefore desirable to record nose-anus length and tail length separately. Hereafter the nose-anus length is referred to as body length.

The difference in body length between control males and females and that between gonadectomised males and females are both significant. The order of absolute body length from greater to smaller is as follows: controls, castrated males, spayed females and control females. However, when the relative length is considered, reverse will be the order.

The castrated males failed to attain the body weight of normal males by 8.75%, yet they only failed to grow 2.56% in body length, which suggests that the general skeletal growth of castrated males is not as much hindered by the operation as the body weight.

The spayed females gained 12.01% more in body weight than their control sisters, but they only surpassed them 2.32% in body length.

Tail length. A significant difference will be seen between control males and females, the latter being 9.02% shorter. After gonadectomy the difference is reduced to only 1.17%. Castrated males are 1.49% smaller in tail length than controls, but this is not significant. Spayed females are significantly longer than controls, with a difference of 7.09%.

Growth of organs

The data obtained on weight of part and organs of body have been studied both as absolute and relative measurements. The object has been to determine whether the effects of gonadectomy on the growth of rats have been produced by a general hastening or retarding of body growth or whether particular organs have been affected more than others. Since the final weights of the animals in groups A and B were similar, the groups have been combined in the following sections of the paper. Tables 4 and 5 give the means, coefficients of variability, and the significances between controls and gonadectomised rats.

A general inspection of table 4 shows that the castrated males were smaller than controls in most of the large organs. The total body weight was significantly less, but the differences between the means for the various parts of the body, with notable exceptions, were not significant. The general tendency for all the larger organs to share the reduced size in castrated animals is however clear. This tendency reaches a high level of significance in respect of the liver. The most noteworthy feature of the table is the increased size of small organs of internal secretion, namely, the hypophysis, the suprarenals and the thymus. The average weights of these organs in the castrated were not only absolutely but relatively considerably larger than those of the controls.

In the females, the larger size of the spayed rats is reflected in most of the organs, but here again some notable departures from this rule occur which involve the endocrine organs. The thymus is again much enlarged, but the suprarenals are relatively much smaller than the controls, and the weight of the hypophysis, although actually larger, is relatively the same weight in spayed and control females.

In contradiction to the males, the liver and kidneys show little difference in actual weight between test and control females.

The coefficients of variability shown in tables 4 and 5 indicate that body weight and length have fairly low variability. Most larger organs are of medium variability, while the lungs, spleen, fat, male accessory organs, uterus, thymus, and suprarenals are of high variability.

Head weight. The difference of head weight between control males and females is 22.56% in favor of the males. The same relation holds after gonadectomy, but the difference is only 8.9%. Control males are not significantly heavier in head weight than castrated. In females, however, the difference between spayed and controls is even more significant than that between castrated males and spayed females.

Skeleton, muscles and spinal cord. A striking difference in the weight of skeleton, muscles and spinal cord is exhibited between control males and females, the former being 46.94% heavier. This difference was reduced to one-third among gonadectomised animals. Castrated males and spayed females have greater relative weight than their respective controls, but the difference is slight.

Integument. Comparing weight within sex, control males are 5.61% heavier than castrated, while spayed females exceed by 17.55% the controls. The latter should be considered very significant, and confirms fully the findings of Freudenberg and his associates ('35, '37, '37).

The relative weights of integuments of all four groups are very similar. Operated males and females are heavier than their controls.

Hypophysis. Among the controls, the females have a 5.85% greater weight of hypophysis than males. Although it is not significant if only the absolute values are compared, however, since the females are much lighter in their body weight than the males, the relative weight of hypophysis for females is much greater than that of males. Castrated rats were 10.38% greater in their hypophysis weight than spayed females, yet owing to wide variations this cannot be considered significant. Test males are 26.64% heavier than controls in hypophysis. Spayed rats are only 7.14% heavier and the difference is not

significant. But if one compares the relative weights in different groups of animals, an astonishing relationship between hypophysis and body weight will be seen. The females attain a weight which is both actually and relatively greater than in the males. Moreover, from comparisons between gonadectomised and control animals it can be seen that the castrated rats grow larger hypophysis than normals, whereas in females the spayed did just reach the relative weight of controls. This seems to show that there is a sex difference in response to gonadectomy so far as the hypophysis is concerned. And this difference may also be attributed for the sex difference in response of body weight growth to gonadectomy. The castrated rats failed to gain as much body weight as the normal males, but they grew a larger hypophysis. The spayed females grew faster than their controls, yet their hypophysis is relatively the same.

With our present incomplete knowledge on the relationship between endocrine function and growth of an animal, we are not yet in the position to interpret the modified growth of the body and hypophysis following gonadectomy. But certain facts may be recalled. First, the association of decreased body weight and hypertrophy of hypophysis, either produced through castration (Hatai, '15; Livingston, '16) or by injection of oestrin (Freudenberger and Clausen, '37) has been generally admitted. Second, the potency of the hypophysis hormones is not positively correlated to gland size (Evans and Simpson, '29). Third, the disturbance of the normal physiological activities in the animals after the removal of gonads may indirectly or directly affect endocrine organs other than the pituitaries, and in attempt to find a solution, they should not be neglected, especially those concerned with metabolism.

Thyroid gland. Though the control males have a 9.6% greater weight for their thyroid gland than female controls, yet it is not significant owing to the wide variations existing. Test males have a 11.9% greater weight than test females, test males 1.8% greater and spayed 1.9% smaller than their respective controls, and are all insignificant. But the normal

variability in the weight of thyroid is great, which renders it difficult to attempt an interpretation of the data available from the present experiment.

Hatai ('15) stated while other endocrine glands all showed their sex difference in size, the thyroid gland was without sex difference. Livingston ('16) observed a moderate decrease of thyroid weight in castrated rabbits. Korenchevsky ('30) noted a 30% lighter average weight of thyroid in castrated rats, but he did not draw conclusions owing no doubt to the wide variations. Lawless ('36) found slight decrease in thyroid weight in most of the castrated rats. Freudenberger and associates ('35, '37, '37) working with female rats found a significant change after spaying in one of their experiments, a probable one in another, yet no change could be detected in the third. The results from their laboratory indicated, they state, that there is an early hypertrophy of thyroid which later disappears. This was said to be also true of the hypophysis.

Submaxillary gland. On the average control males have a 22.4% greater submaxillary gland weight than control females. Between test males and females the difference is 12.05%. No significant difference is found between treated and control animals within their own sexes.

Thymus. Castrated male rats showed a greater thymus weight of 46.3% than that of controls; the spayed weighed 31.3% more than normal females. The thymus weight of female controls was found 3.9% greater than that of male controls, but after spaying the females are 6.75% lighter in their thymus than castrated males.

Since the involution of thymus closely corresponds with time of puberty, it is conceivable that as soon as the gonads are removed this natural process will henceforth be postponed. The thymus is not altogether a lymphoid organ, but is also concerned with nutrition, growth and balance of the endocrine activities. Freudenberger and Clausen ('37) observed that the thymus became smaller in oestrin-injected female rats before the body weight decreased. This led them to suspect the possibility that the thymus might be responsible for the

smaller body weight in treated animals. This supposition does not appear to allow for involution of thymus in normal growing animals.

Spleen. A difference of 18.21% was found between control males and females favorable to the former. Among the test animals the difference becomes 15.19%. An increase of 5.14% for castrated males and 1.39% for spayed was indicated from a comparison with their respective controls. As the spleen is a very variable organ, these differences may mean very little.

Suprarenal glands. The weight of suprarenal glands of female rats is strikingly greater than that of males, in spite of the fact that the latter are greater in body weight. This difference is 57.4% in favor of control females, and 19.5% for spayed females. Castrated males gained 16.6% over normals, while spayed females showed a decrease of 11.4% compared with controls. These differences are all significant.

The decreased body weight of castrated males is associated with a significant increase in suprarenal weight, whereas the spayed females show a smaller suprarenal weight than normal female rats. Since the gland is concerned with both sexual activity and metabolism, its differential response to the removal of testes in males and ovaries in females cannot be overlooked.

The greater suprarenal weight is established in both groups of castrated males. The difference reached was more pronounced in those animals castrated at an older age, the difference of group B amounted to more than three times greater than that between group A animals. Group A spayed females have a suprarenal weight about 10% less than that of controls, the difference becomes 14.6% in group B females. It appears that gonadectomy on sexually matured animals has a greater influence upon suprarenal glands than on younger ones.

An interesting feature of the response of the suprarenals to gonadectomy is its resemblance to that of the hypophysis. In both cases, the glands became larger following castration. After spaying, the suprarenals became smaller, while in the hypophysis no change in the relative weight was found,

although others have found it. This similarity in response indicates that their functions are closely linked.

Stomach and intestines. It is clearly indicated that the weights of stomach and intestines are closely associated with the size of the animals. The larger the size of an animal depends on a larger capacity for food consumption and utilization, or equally well the relation may be inverted or both may depend on a growth or size factor.

Fat. Only the gross visible fat from the renal and inguinal regions as well as subcutaneous fat was weighed. An increase of 4.2% was found in castrates as compared with normal males. The spayed females gained 22.53% more than controls. None of these differences are significant owing to the great variation. Nevertheless, it suggests that spaying does tend to increase the amount of fat.

Sexual organs. The hypotrophy of the male accessory organs in castrated rats is almost complete. The organs affected are the ductus deferens, the prostate gland, the seminal vesicles, and the coagulating glands attached to them. The failure of development of these organs has been shown to be due to the absence of testes hormones. In fact, modern methods of assay of hormone from testes utilized this reaction.

Uterus in spayed females underwent the same fate as the male accessories.

Bone growth. An attempt was made to determine whether the skeletal growth was affected by the removal of the sex glands; if it is, it would be interesting to know to what extent it is affected. Bones from eight litters of rats were used, including fifteen normal males, fifteen castrated males, sixteen female controls and sixteen spayed females.

a. Measurement of tibia and hip bone lengths: The results of the measurement are summarized as follows:

TABLE 7
Mean lengths of tibia and hip bone in centimeters

	MALE RATS		FEMALE RATS	
	Controls	Castrated	Controls	Spayed
Tibiae	3.64	3.57	3.31	3.40
Hip bones	3.98	3.78	3.61	3.66

On the average the normal male controls have a 2% greater tibiae and 5% greater hip bones length than castrated litter-mates. On the other hand, the spayed females have an excess of tibiae length of 2.6% and of hip bones length of 1.3%. These differences are not very large, but indicate that the smaller body weight of castrated males than that of controls and the

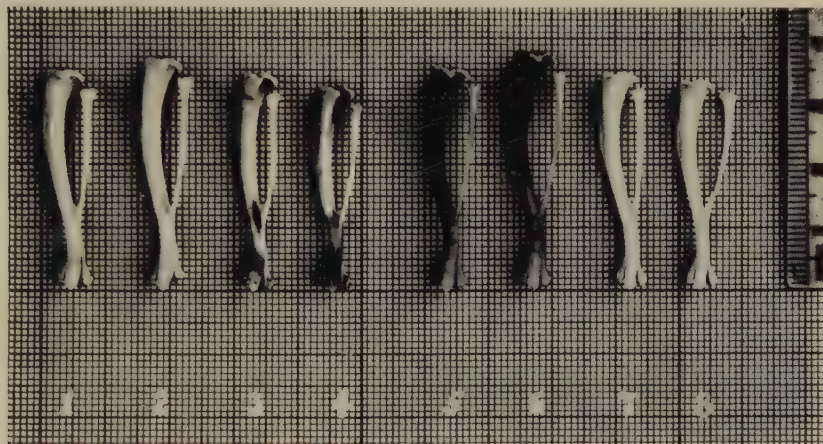


Fig. 2 Tibiae and fibulae from control and gonadectomised rats. (See below.)

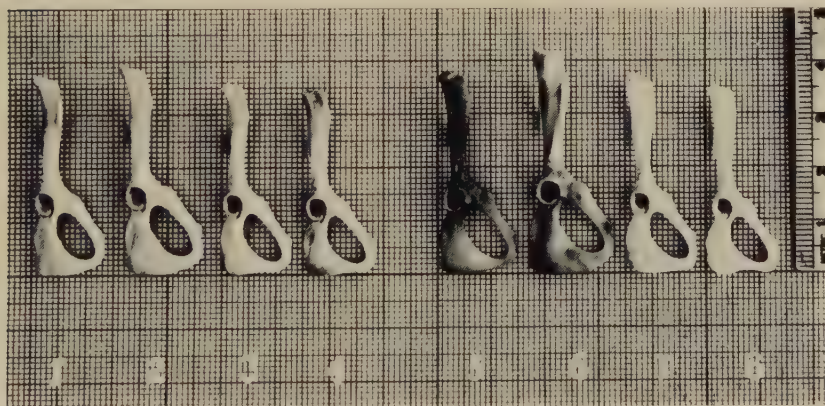


Fig. 3 Hip bones from control and gonadectomised rats.

1, 5, castrated males (rat nos. 43, 110).

2, 6, control males (rat nos. 42, 111).

3, 7, spayed females (rat nos. 45, 89).

4, 8, control females (rat nos. 48, 88).

greater weight of spayed females than their controls are based in part at least upon the differences in skeletal development.

It is interesting to note from figures 2 and 3 how the sex difference has been greatly reduced by gonadectomy in the growth of tibiae and hip bones.

b. Examination of epiphyseal union: Examination of the stages of epiphyseal union has been made. Apparently the animals used in the present investigation were sacrificed too young, for no differences were detected either between test and control rats or between males and females. Epiphyseal union had already taken place at: (1) capitellum and trochlea; (2) proximal radius; (3) distal tibia; (4) tuberosity of calcaneum; (5) distal fibula; (6) head of scapula; (7) hip bone (primary elements). Epiphyses not united were: (1) medial epicondyle of humerus; (2) olecranon of ulna; (3) head of femur; (4) greater trochanter; (5) lesser trochanter; (6) distal femur; (7) proximal tibia; (8) proximal fibula; (9) distal radius; (10) distal ulna; (11) head of humerus; (12) base of coracoid.

SUMMARY AND CONCLUSIONS

1. A total of seventy-five albino rats of both sexes was used in this experiment, comprising thirty-six gonadectomised and thirty-nine control animals. Incisions were made on controls and their gonads were examined but kept intact.

2. Growth rate and amount of food consumed were recorded for 10 weeks. All the animals were autopsied at the end of the experiment.

3. The castrated males grew on a slower rate than their controls, while spayed females grew faster than their normal littermate sisters; thus resulted in an intermediate type of growth curves for gonadectomised males and females.

4. In evaluating the efficiency of food utilization of test and control animals, efficiency quotients (E.Q.) were calculated by the following formula:

$$\text{E.Q.} = \frac{\text{gain in weight (grams)}}{\text{food consumption (grams)}} \cdot \frac{\text{initial weight} + \frac{1}{2} \text{ gain}}{100}$$

The E.Q. values for the various groups of animals were: control males, 0.220; castrated males, 0.196; spayed females, 0.155; control females, 0.125. When the differences between test and controls are expressed in percentage of controls, castration reduced efficiency by 7%, spaying increased efficiency by 31%. Gonadectomy reduced the sex difference in efficiency from 43% among controls to 19% in test animals.

5. Both the castrated and spayed rats consumed less food than their respective controls.

6. Castrated rats had a body weight 8.8% lighter and spayed females 12% heavier than their respective controls; both differences were significant. The removal of gonads reduced the sex difference in body weight from 29.6% to 13.6%.

7. Comparisons of organ growth were made between test and control rats, between control males and females and between test males and females.

8. A sex difference in regard to the response of hypophysis and suprarenals to gonadectomy seemed to be definitely established. In both cases hypertrophy of the organs was accompanied by a decreased body weight.

9. Thymus gland weight was very significantly greater in test males and females than their controls.

10. Weight of kidneys in castrated males was significantly heavier and liver weight significantly lighter than control males; while in females, spayed had significantly greater body and tail lengths, heavier skeleton, muscles and spinal cord, integument, heart and stomach. The remaining genital tract in both castrated and spayed rats greatly atrophied.

11. The coefficients of variability showed that lungs, spleen, fat, thymus, suprarenals and genital organs were very variable organs; other organs were of medium variability, and total body weight and nose-anus length of low variability.

12. From the measurement of the lengths of body, tail, tibia, and of hip bone it indicated that the smaller body weight of castrated than that of control males and greater weight of spayed than that of control females were due in part at least to the differences in skeletal development.

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STUDIES ON AN ANOPHTHALMIC STRAIN OF MICE

II. EFFECT OF CONGENITAL EYELESSNESS ON REPRODUCTIVE PHENOMENA

ELIZABETH BROWN CHASE

Department of Zoology, University of Illinois, Urbana

Studies have been made of the effect of enucleation upon maturity and oestrous cycles in some polyoestrous rodents. There has been no consideration, however, of a congenitally anophthalmic group. The present study was undertaken to see if an eyeless strain of mice differed from a normal strain in various reproductive phenomena.

MATERIALS AND METHODS

In the anophthalmic mice used in the present study, the young are born with no eyes or eye remnants and with no optic nerves (Chase, '41; Chase and Chase, '41). The highly inbred C57 Black strain was used for comparison. All animals were kept under identical conditions of caging and feeding in our colony. The observations were made in the months from November to June.

The females were kept at all times with littermate brothers or with tested males. Beginning at 22 days of age, examinations were made daily and the age at introitus and the age at which the first litter was born were recorded. In addition, daily vaginal smears were made of some of the animals from the time the vagina opened until the first litter was born. Sections of uterus and vagina and serial sections of ovaries of seven normal females and six blind females at introitus were studied for possible histological differences at that time.

OBSERVATIONS ON REPRODUCTIVE PHENOMENA

The results of the study of female mice are given in table 1. The age at introitus is slightly earlier in the eyeless mice. This difference may be significant as there is a probability of 0.03

of getting as bad or worse results in another comparison of samples. Serial sections of ovaries taken when the vagina opened show follicles of medium size in both strains, with no large follicles and no corpora lutea. At this time the uterus and vagina are very small. The first cornified vaginal smear was found some days after introitus in both groups. The age at which this appeared was significantly earlier in the eyeless than in the control females. The number of cornified smears before the first fertile mating is not significantly different in these strains. A comparison of the age at which the first litters were born shows no significant difference between the

TABLE 1
Comparison of reproduction in females

STRAIN	AGE AT INTROITUS		AGE AT FIRST CORNIFIED SMEAR		NUMBER CORN. SMEARS BEFORE FIRST LITTER		AGE AT FIRST LITTER			
	n	Mean ± S.E.	n	Mean ± S.E.	n	Mean ± S.E.	Littermate matings		Tested male matings	
C57 Black	167	34.53 ±0.45	40	53.20 ±1.84	38 ¹	2.79 ±0.36	25	78.36 ±2.02	49	91.73 ±2.42
Eyeless	79	32.84 ±0.64	22	42.82 ±2.15	21 ²	2.24 ±0.69	22	80.23 ±2.45	24	92.54 ±3.47
Probability of difference		0.03	0.00024		0.48		0.56		0.85	

¹ Two animals died after mating with older males.

² One animal had normal cycles as shown by vaginal smears but had no litters.

normal and eyeless females either in the case of females placed with littermate brothers or in those placed with older males.

The oestrous cycles as shown by vaginal smears were irregular in both strains, but no more so in one than in the other. The average length of cycle did not differ significantly in the two groups, as shown in table 2. The number of fertile matings at the first oestrus was 35.9% for the normals (forty animals) and 27.3% for the eyeless mice (twenty-two animals).

The anophthalmic males mature as early as the normal. In table 3 are given some observations of sperm motility in epididymal smears from males 35 to 45 days of age. There is considerable variability in this regard but no real difference

TABLE 2
Average length of oestrous cycles

STRAIN	NO. ANIMALS	NO. CYCLES	LENGTH OF CYCLE MEAN $\pm$ S.E.	PROBABILITY OF DIFFERENCE
C57 Black	26	71	6.90 $\pm$ 1.37	0.56
Eyeless	16	51	8.39 $\pm$ 2.23	

TABLE 3
Motile sperm in epididymal smears

STRAIN	35 DAYS		40 DAYS		45 DAYS	
	n	Number with motile sperm	n	Number with motile sperm	n	Number with motile sperm
C57 Black	13	2	19	16	14	12
Eyeless	12	1	9	6	13	12

between strains. The earliest age at which litters were born to littermate brother-sister matings was 61 days for the controls and 64 days for the blind mice.

DISCUSSION

In this study, the blind animals have shown no retardation of sexual development as compared with the normal black mice. In fact, the only definitely significant difference between the two groups was in the age at which the first cornified vaginal smear appeared, when the eyeless animals were significantly younger than the controls. The age at introitus, frequently used as an index of maturity, may be significantly different in the two lines. However, if this is considered significant, it shows an earlier introitus for the anophthalmic animals. The age at which the first litter was born was not different for the two strains. The males mature at about the same age in both strains. These findings are interesting in comparison with those of Browman ('38, '40) who found a definite retardation of maturity in rats enucleated at birth.

The oestrous cycles were irregular in both strains of mice, but the average length of cycle was not significantly different. Browman ('37) found that oestrous cycles were normal in rats enucleated when mature. In *Peromyscus*, blinded animals have been found to have no cyclic tendency to breeding activity but are not sterile (Whitaker, '40).

Although there is a disadvantage in this study in that the blind animals and the normal ones are of different inbred strains, study of the very few anophthalmic animals which occasionally appear in the C57 Black strain show that they do not differ in reproductive phenomena from the normal blacks. At present it can be said that in the house mouse, the eyes and optic nerves are not necessary for normal maturation, oestrous cycles, mating, or production of young.

SUMMARY AND CONCLUSIONS

1. A congenitally anophthalmic strain of mice was compared with a normal strain in regard to reproductive phenomena.

2. The eyeless mice were possibly significantly younger on the average at the time of introitus and definitely younger at the time of the first cornified vaginal smear. There was no significant difference in the length of cycle, in the number of cornified smears before a fertile mating, or in the age at which the first litter was produced.

3. Histological study of ovaries, uterus, and vagina at introitus showed no difference in the two strains.

4. The males of both strains mature at about the same age as shown by epididymal smears and by the ages when first litters were produced.

5. Normal reproductive phenomena in the house mouse occur in the absence of eyes and optic nerves.

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THE VAGINAL SMEAR PICTURE, SEXUAL RECEPTIVITY AND TIME OF OVULA- TION IN THE ALBINO RAT ¹

WILLIAM C. YOUNG, JOHN L. BOLING AND RICHARD J. BLANDAU

*Laboratories of Primate Biology, Yale University School of Medicine,
New Haven, Connecticut, and Arnold Biological Laboratory,
Brown University, Providence, Rhode Island*

The sudden acceleration in the rate of graafian follicle growth which coincides with the beginning of heat in the guinea pig and rat (Myers, Young and Dempsey, '36; Boling, Blandau, Soderwall and Young, '41) marks this point as one of considerable importance in the estrous cycle of these species. Indirect evidence suggests that the first effects of progesterone are manifest about this time (Dempsey, Hertz and Young, '36; Astwood, '39) even though the follicle has not yet ruptured. It seemed probable, therefore, that this end-point may have considerable usefulness for a variety of investigations for which the rat would be chosen even as it has been utilized in the guinea pig (Dempsey, '37; Collins, Boling, Dempsey and Young, '38; Blandau and Young, '39; Boling, Blandau, Wilson and Young, '39; Soderwall and Young, '40). Such being the case, the establishment of an accurate method for identifying the "moment" the preovulatory swelling begins was considered desirable.

The opinion is general that the beginning of heat which marks this point in the cycle is easily determined by reference to the vaginal smear picture, that it usually coincides with the beginning of complete cornification (Long and Evans, '22).

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Were this invariably true, reference to the vaginal condition would also be sufficient for the identification of the beginning of the preovulatory swelling. Experience with the guinea pig has shown, however, that the relationship between the vaginal condition and the beginning of heat is not constant (Fellner, '32; Young, '37); consequently, identification of the beginning of heat is more certain when reliance is placed on the ability to elicit the copulatory response.

The possibility that this might also be true in the rat was suggested by the observation that in this species too, the relationship between heat and the vaginal condition varies (Ishii, '22; Long and Evans, '22; Smith and Engle, '27; Hemmingsen, '33; Ball, '37). Were this variation slight or of infrequent occurrence, the beginning of heat might still be determined from the vaginal condition with a degree of accuracy that would be sufficient for most practical purposes. There are indications, however, that the variation is considerable (Hemmingsen, '33; Ball, '37) and that reference to the behavior is more dependable.

The same possibility existed with respect to the relationship between the vaginal condition and ovulation. Long and Evans concluded that ovulation occurs during the last hours of the cornified cell stage, but there is no evidence that the relationship is constant. More important, however, was the likelihood that the relationship might differ from that which they postulated. When they concluded that ovulation occurs toward the end of the stage of cornification it was assumed that ovulation takes place at a considerable interval after the end of heat. In our experience, however, ovulation usually occurs before the end of heat (Boling, Blandau, Soderwall and Young, '41). If this is true, ovulation should occur earlier than the late cornified cell stage.

Reinvestigation of this relationship and that between the vaginal condition and heat was undertaken during observations on the growth of the graafian follicle (Boling, Blandau, Soderwall and Young, '41) and the length of heat (Blandau, Boling and Young, '41).

MATERIALS AND METHODS

The vaginal smears were obtained during a period when 145 female albino rats were being observed at hourly intervals, day and night, under conditions which had no harmful action on the regularity or character of the estrous cycle (Blandau, Boling and Young, '41). One hundred and thirty-six sets of smears were made from fifty-three normal animals. Each set was composed of two smears, one made within an hour of the beginning of heat determined by the copulatory response and the other made within an hour after the end of heat. Thirty-nine single smears were made near the time of ovulation. Of these, fifteen were made before any follicle had ruptured, but not less than 7 hours after the beginning of heat. Twenty-four were made after at least one follicle had ruptured or after ovulation was complete, but in no instance more than 12 hours after the beginning of heat. All smears were allowed to dry and were then stained with Delafield's hematoxylin and eosin.

When the senior author was investigating the relationship between the vaginal condition, sexual receptivity and ovulation in the guinea pig (Young, '37), he had the choice of accepting the terminology of Stockard and Papanicolaou ('17) and considering the period when epithelial and cornified cells predominate as stage 1, or adopting the terminology used by Long and Evans ('22) in which stage 1 is the period during which nucleated epithelial cells predominate and stage 2 is the period when cornified cells predominate. The former alternative was chosen in order that the new work could be kept as nearly as possible comparable with the old. In this study the same rule has been followed, but with the unfortunate result that the terminologies employed in the comparative studies of the rat and guinea pig are not identical. In a general way the various parts of stage 1 in the investigation of the guinea pig correspond to stages 1 and 2 in this study, stage 2 in the study of the guinea pig corresponds to stage 3 in this study, and stage 3 of the older investigation corresponds to stage 4 in this.

OBSERVATIONS

The sequence of cell types found in the vaginal smears was not different from that described by Long and Evans although it is thought that the non-cornified epithelial cells which they describe as reappearing among the cornified cells and leucocytes should be given more emphasis. In our preparations these cells usually replace the cornified cells prior to leucocytic infiltration. With Selle ('22) we regard them as homologous with the cells composing Stockard and Papanicolaou's stage 2 which are subsequently referred to as "pavement" cells (Papanicolaou, '33). Because of the completeness with which these cells replace the cornified cells we have referred to the short period when they predominate as stage 3.

The essential data bearing on the relationship between the vaginal condition on the one hand, and the time of heat and ovulation on the other, are summarized in table 1. Their significance is clearest when they are compared with those presented by Long and Evans, Hemmingsen, and Ball.

TABLE 1

The relationship of the vaginal smear picture to the time of heat and ovulation

STAGE	CELL-TYPES	BEGIN- NING OF HEAT	END OF HEAT	OVULATION		CORRE- SPONDING STAGE OF LONG AND EVANS
				No rup- tured follicles	In prog- ress or complete	
1	Nucleated epithelial cells only	5	0	0	0	1
	Nucleated epithelial cells, 75%; cornified cells, 25%	96	0	4	0	
	Nucleated epithelial cells, 50%; cornified cells, 50%	13	0	3	0	
	Nucleated epithelial cells, 25%; cornified cells, 75%	6	3	4	4	
2	Cornified cells only	9	19	3	12	2 and 3
	Cornified cells, 75%; "pavement" cells, 25%	0	27	0	4	
	Cornified cells, 50%; "pavement" cells, 50%	2	27	0	1	
	Cornified cells, 25%; "pavement" cells, 75%	4	50	1	3	
3	"Pavement" cells only	1	7	0	0	
4	"Pavement" cells and leucocytes	0	3	0	0	4

All are agreed that the time of mating may vary with respect to the vaginal condition, but it seems clear from the Long and Evans and Ball monographs that the first unmistakable signs of heat coincide with the beginning of complete cornification more often than with any other vaginal condition. On the other hand, analysis of the cycles considered by Hemmingsen to be most typical shows that a strong intensity of heat was being displayed prior to the time of complete cornification. The latter results are in agreement with those summarized in table 1 which indicate that in the animals we studied the beginning of heat tended to coincide with the first appearance of cornified cells rather than with complete cornification; indeed, by the time cornification was complete heat had ended in a few animals.

If this relationship is the one encountered most commonly in the rat, the period of cornification is not the period of heat in the sense that heat usually begins at this time. The vaginal condition with which the beginning of heat, and therefore the beginning of the preovulatory swelling, tends to be associated is that characteristic of the time when the first cornified cells appear. This stage may precede complete cornification by an average of 8 to 11 hours, an estimate which is based on the average length of heat in twenty-two animals which came into heat when 75% or more of the cells were nucleated epithelial elements and went out of heat either when 75% of the cells were cornified or when cornification was complete. If the estimate is correct, it appears that in most animals a very considerable portion of the heat period has elapsed before cornification is complete. Verification would seem to be given by Hemmingsen's observation that the transition from the stage of nucleated epithelial cells to cornified cells is gradual and ordinarily occurs between noon and midnight. Consequently, while the period of complete cornification is a likely time to detect heat when a "cross-section" is desired, it should be recognized that much of estrus may have elapsed by this time. Also to be remembered in this connection is the fact that cornification is sometimes seen in the absence of heat

(Hemmingsen, '33; Witschi and Pfeiffer, '35; Ball, '37; Boling, Blandau, Rundlett and Young, '41).

The end of heat is not associated as closely with any one vaginal condition as the beginning. This may be attributable to the circumstance that the length of heat is so variable. It is clear, however, that heat ends in most animals, not only after cornification is complete, but usually after the nucleated epithelial cells have reappeared.

The data bearing on the relationship between the vaginal condition and ovulation are also summarized in table 1. They do not support the opinion that ovulation occurs during the last hours of the cornified cell stage. On the contrary, they indicate that ovulation ordinarily begins and is completed much earlier than this. Of the four animals in which ovulation had not occurred by this time, it was imminent in all; in fact, in one animal the first follicle had ruptured, but the egg had not reached the opening through which the follicular fluid was flowing (Boling, Blandau, Soderwall and Young, '41, fig. 9). In eight of twelve animals in which cornified cells only were seen, ovulation was complete and of the eight killed after nucleated epithelial cells had again appeared, ovulation was complete in six.

The conclusion that ovulation usually occurs early in the stage of cornification is consistent with the data bearing on the relationship between ovulation and the time of heat (Boling, Blandau, Soderwall and Young, '41). Forty-two animals were killed in which rupture of the follicles was occurring or was complete. Of these, thirty-one were still in heat. In view of the fact that ovulation usually takes place before the end of heat it is not surprising that the vaginal condition at the time of ovulation is less advanced than that at the end of heat.

DISCUSSION

Not all the differences between the data presented above and those which have been reported previously are easy to account for. Chief among these are the relationship between the beginning of heat and the vaginal condition and that

between the time of ovulation and the vaginal condition. In all likelihood a combination of factors is involved. When the earlier studies were made, investigators had but slight familiarity with the external signs of heat. It is possible therefore that accurate identification of the beginning of heat was not often achieved. Observations were less frequent and when they were made as often as at 3-hour intervals (Ball, '37), abnormalities appeared after the first cycle which were reflected by prolonged periods of cornification and longer estrous cycles. Finally, allowance must be made for the possibility that a "vaginal estrus" was sometimes taken for a true estrus. The latter was considered difficult to identify and the discovery that certain vaginal conditions usually are associated with the occurrence of heat was grasped as the way out of a difficulty which had long presented obstacles to large-scale experimentation.

In many ways the relationships are similar to those seen in the guinea pig. The progression of cell-types is approximately the same. In the rat as in the guinea pig (Stockard and Papanicolaou, '19; Genter, '34; Young, '37) the beginning of heat is associated with the first appearance of cornified cells. In the rat, on the other hand, the end of heat tends to occur later with respect to the vaginal condition. In most rats it coincides with the reappearance of the nucleated epithelial cells whereas in most guinea pigs the end is associated with an earlier stage when large numbers of cornified cells are just appearing (Young, '37). In the guinea pig ovulation usually occurs before cornification is complete. In the rat also ovulation often has this relationship to the vaginal condition, but more commonly in the animals we have studied, it coincides with the beginning of complete cornification.

SUMMARY AND CONCLUSIONS

The relationship of the vaginal condition to the beginning of heat, marking the beginning of the preovulatory swelling, to the end of heat and to the time of ovulation has been investigated in a group of normal adult female rats.

1. The relationship between the willingness to mate and the vaginal condition is not constant, but heat begins in most animals about the time the first cornified cells appear rather than about the time cornification becomes complete.

2. The end of heat is associated less closely with any one vaginal condition, but in most animals it occurs after the period of cornification when nucleated epithelial cells are reappearing.

3. The time of ovulation varies with respect to the vaginal condition, but in most animals it takes place about the time cornification is complete rather than later in the stage of cornification.

4. Identification of such end-points as the beginning and end of heat, the beginning of the preovulatory swelling and the time of ovulation is generally more accurate when reference is made to the behavioral responses associated with estrus rather than to the vaginal condition.

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A CASE OF TRUE HERMAPHRODITISM IN THE FIELD MOUSE

S. A. ASDELL, W. J. HAMILTON, JR. AND K. P. HUMMEL
*Laboratory of Animal Nutrition and Department of Zoology, Cornell University,
Ithaca, New York*

THREE FIGURES

On August 25, 1936, one of us (W. J. H.) trapped at Ithaca, New York, an adult field mouse, *Microtus pennsylvanicus pennsylvanicus* (Ord), which from external appearances was classified as a male. On dissection, however, a uterus was found, so a thorough search was made, revealing the fact that the left gonad was a testis, while the right was an ovary. As hermaphroditism in wild animals has been very infrequently reported, a detailed description of this case is given.

The only cases of hermaphroditism of wild mammals which we have found in the literature are the following:

1. Brambell and Hall ('35-'36), pseudohermaphroditism in *Sorex minutus*.

2. Hartman and League ('25), pseudohermaphroditism in the opossum (*Didelphys virginiana*). This case was peculiar, as ovaries were present instead of testes, which are usual in pseudohermaphrodites.

3. L. J. Wells' ('36-'37) two cases of true hermaphroditism in ground animals (*Citellus tridecemlineatus*). These animals had bilateral ovotestes.

4. Brown ('23-'24), hermaphroditism in the mole (*Talpa europaea*). An ovotestis was found on one side; the rest of the specimen was incomplete.

We have found three recorded cases of hermaphroditism in the Muridae, all in the laboratory mouse (*Mus musculus*).

1. Blotvogel's ('32) case. This was a mouse with female external genitalia. The right gonad was an ovary and the left a testis. The accessory structures were all female and fully developed. No male accessory organs were found. This case is incorrectly described as one of hermaphroditismus verus unilateralis; it should be hermaphroditismus verus lateralis.

2. Danforth's ('27) mouse. The left gonad was an ovary. The right was a testis. Externally the specimen resembled a female. The uterine body and left cornu were normal, but on the right side the ductus deferens and vesicula seminalis were normal. There was complete partition of male structures to the right side and female to the left side of the body, except for a few prostatic ducts on the female side. Danforth interpreted this case as a gynandromorph.

3. Fekete's ('37) case. This was a mouse with a normal ovary, oviduct and uterine horn on the right side, and a testis, epididymis, ductus deferens and seminal vesicle on the left side, together with a truncated uterus. The external genitalia were female.

Two cases of true hermaphroditism in rats (*Mus rattus*) may be added to these instances. These have been found in laboratory rats by Dr. R. O. Greep. The description of these animals has not yet been published.

THE PRESENT CASE

The following is a description of the specimen of *Microtus pennsylvanicus* which is the subject of this paper.

A. Male structures

Left gonad. This is a testis, surrounded by fat. The seminiferous tubules have a single layer of large cells at their peripheries. No mitoses are present. The lumina are filled with what appears to be coagulated tissue fluids. Interstitial cells are present. The size of the tubules and the amount of interstitial tissue are approximately normal. The condition of the tubules is that usually found in the cryptorchid.

Epididymis. This is attached in the normal way to the testis. It was entirely surrounded by fat which was dissected off to reveal the connection with the testis. The epithelium is columnar with small cilia. The height is even, and the lumen presents an even section throughout its length. Some cellular debris are present, but no spermatozoa. There is very little connective tissue between the tubules.

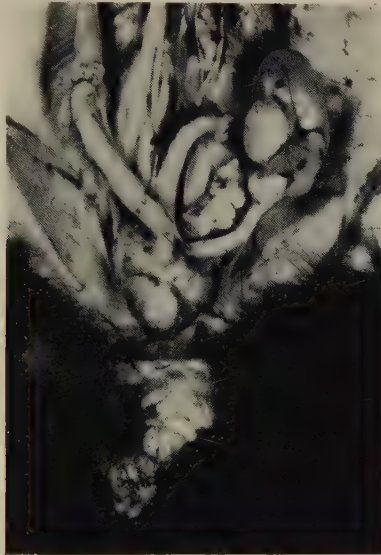


Fig. 1 Dissection of hermaphrodite *Microtus pennsylvanicus* showing on the right of the illustration testis, epididymis (enclosed in fat), ductus deferens, vesicula seminalis and prostate. On the left, ovary, oviduct and uterus.

Ductus deferens. This tube is normal on the left side, except for what appeared on gross dissection to be a blind diverticulum at the junction with the epididymis. On sectioning, this was found to be a much coiled portion continuous with the vas. The wall of both parts is normal in structure, but the epithelium is of uneven height. The ductus deferens enters the urethra at approximately the same level as the prostate.

Prostate. This organ is normal in its histological appearance. Secretion is present in some of the tubules. It connects with the urethra by a number of tubules.

Vesicula seminalis. This is normal in histological appearance. It opens into the urethra between the prostate and the vagina.

The prostate and vesicula seminalis form a complex mass around the urethra at the base of the bladder and the uterine body. The vesicula seminalis lies mainly dorsal to the uterus and bladder on the left side. On the right it is more compact and has been pushed to one side posteriorly by the uterine horn. No separate seminal vesicle was found on the right side.

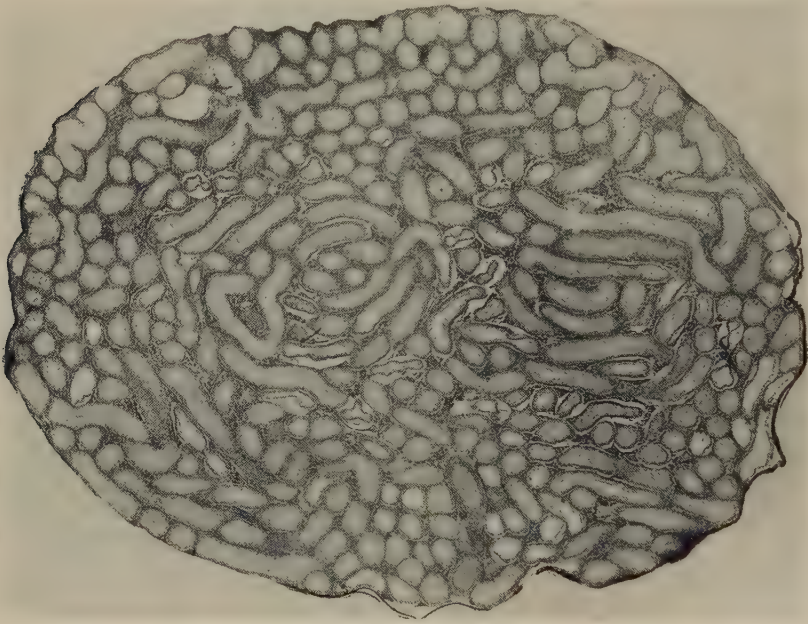
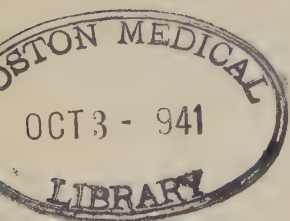


Fig. 2 Testis of hermaphrodite *Microtus pennsylvanicus*. $\times 25$.

Urethra and penis. The penis is quite prominent externally and is rather unusually blunt. It is open on the inferior aspect. The urethra is continuous with this open portion, and is entered in order from the bladder by prostatic tubules, ductus deferens of the left side (that of the right side could not be found), vesicula seminalis and vagina. Serial drawings were made to establish these relationships.



B. Female structures

Ovary. The right gonad is an ovary. Primary ova in nests are present, also young and more mature graafian follicles. Some follicles are large enough for rupture, but the nuclei of the ova are in the resting state. No corpora lutea are present. A few follicles are in the process of degeneration. In one follicle the ovum has divided so that there are three cells, one large and the others slightly smaller. All are nucleated

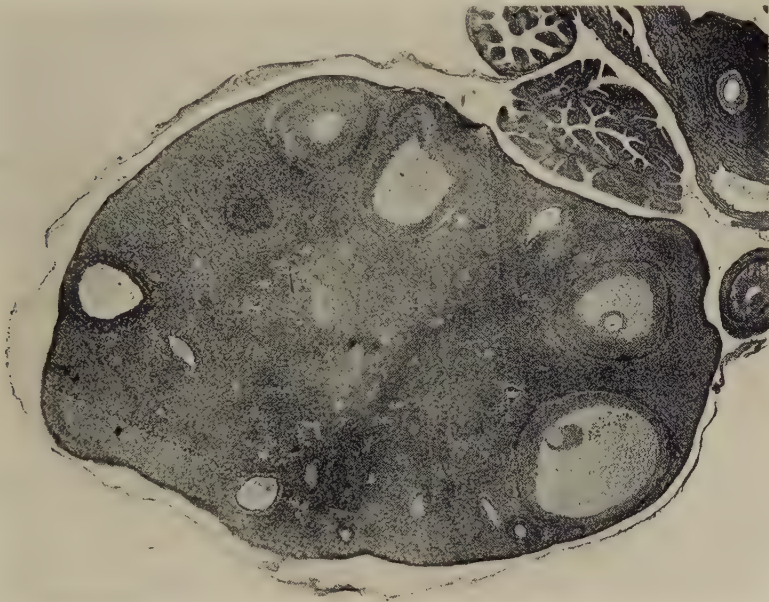


Fig. 3 Ovary of hermaphrodite *Microtus pennsylvanicus*. $\times 25$.

and appear to be healthy. The granulosa in this follicle, which is a large one, shows signs of degeneration, and the ovum itself is not surrounded by granulosa cells. In two other follicles in which the degeneration of the granulosa is further advanced the division or fragmentation has also gone further, so that the resulting state might be described as a morula stage. The cells are all nucleated and are resting, but are very small. Similar conditions in the guinea pig have occasionally been described (e.g., Loeb, '31-'32) as intrafollicular partheno-

genesis, but Harman and Kirgis ('38) interpret the condition as part of the normal process of follicular degeneration in that species. In some of the moderate-sized follicles mitotic activity is present in the granulosa layer.

Oviduct. The oviduct is normal, with ciliated epithelium rather uneven in height. Goblet cells show secretory activity.

Uterus. The uterus is normal and well developed on the right side, with very even low columnar epithelium. Glands are abundant. They possess large lumina. On the left side there is no evidence of uterus or oviduct.

Vagina. The uterus opens into the right side of the vagina, which is lined with stratified squamous epithelium with a heavy cornified coat, which is becoming detached. Leucocytic invasion of the epithelium is in progress and leucocytes are free in the lumen. The picture is that of early metestrus.

The vagina opens into the urethra posterior to the entrance of the seminal vesicles.

DISCUSSION

The case of true hermaphroditism described is definitely of the type often described as a gynandromorph, though this term for "hermaphroditismus verus lateralis" with corresponding accessory organs should, in our opinion be discarded and the term "sex mosaic" which is used for similar cases in insects be substituted. In insects, however, there is definite evidence that many of these sex mosaics differ in their chromosomal constitution in the male and female portions and there is, as yet, no evidence of such a difference in these mammalian forms. The rarity of the condition in the wild state is noteworthy but this may be due to the much closer scrutiny to which laboratory animals are subjected. However, rodents in general seem to be much less liable to the condition than certain other species, notably the pig, goat and possibly man. It should be noted also that this sex mosaic condition has not, so far as we are aware, been described in any other mammalian order than the rodents. Besides the cases already mentioned, there is also the guinea pig described

by Jaffe and Papanicolaou ('27) which falls into this case. In all other orders there is no clear limitation of the male and female accessories to each side of the body corresponding to the nature of the gonad. Instead, male and female accessory organs, in different degrees of development, it is true, are found on the same side.

SUMMARY

A case of true lateral hermaphroditism or of gynandromorphism in *Microtus pennsylvanicus* is described. The accessory sex organs were entirely male on the side of the testis and entirely female on the side of the ovary. This condition, in mammals, has been described only in rodents.

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REPORT OF AN UNUSUAL COELIACO-MESENTERIC TRUNK WITH UNIQUE DISTRIBUTION AND ANASTOMOSES

ROBERT S. MUNGER

*Department of Gross Anatomy, The Tulane University of Louisiana,
New Orleans, Louisiana*

ONE FIGURE

The truncus coeliaco-mesentericus, or coeliaco-mesenteric trunk, is formed by the superior mesenteric artery coming off the aorta in a common stem with the coeliac artery. This is an altogether different, fundamental anatomical type than any described for the coeliac artery per se (Lipshutz, '17). Under the designation coeliaco-mesenteric trunk is to be understood the complete one, that is, one which gives off four arteries, the left gastric, splenic, and hepatic, and superior mesenteric. In the complete trunk, the form of division of the trunk is not considered (Eaton, '17). According to Ruggles ('35), the mean distance between the origins of the coeliac artery and the superior mesenteric artery in man is 1.6 cm., but in a large number of lower animals the common origin of the two arteries is an entirely normal condition (Meckel, 1832; Lipshutz, '17). Adachi, in 1928, collecting the instances reported by Europeans and Americans in a large series of cadavers, reveals that the coeliaco-mesenteric trunk occurred in approximately 1.3%; whereas in his own series of Japanese cadavers, he found it in about 2.4%. Tandler ('04) believes that the formation of the coeliaco-mesenteric trunk results from a persistence of a normal embryological condition, namely, the many-rooted ventral anastomosis involving the early segmental coeliac axis and superior mesenteric groups. Usually the superior mesenteric constitutes the main part of the trunk in this "ventrale Längsanastomose."

DESCRIPTION

The coeliaco-mesenteric trunk, and the distribution and anastomoses of its branches, described herein was found in the cadaver of a middle-aged, female negro. The trunk is a complete one (fig. 1), composed of the above-mentioned essential arteries. Three of them, the splenic, hepatic, and left gastric arteries, branch from a common stem (coeliac artery), and this stem and the fourth artery (superior mesenteric), branch from a common stem again, the coeliaco-mesenteric trunk, which arises directly from the aorta and projects forward above the upper border of the pancreas. Besides these essential arteries, there are two additional arteries: a supernumerary artery; and a common stem for the two inferior phrenic arteries.

As is to be expected from a consideration of the embryological origin of the coeliaco-mesenteric trunk, the superior mesenteric artery is the dominant artery in this specimen. The length of the trunk, measured from its aortic origin to the (distal) exit-angle of the superior mesenteric artery, is 1.0 cm. The left gastric artery comes off the superior aspect of the coeliac artery as a collateral branch immediately after the bifurcation of the truncus. The coeliac artery terminates by dividing into the splenic and hepatic arteries 1.3 cm. from the exit-angle of the superior mesenteric artery. The courses of the superior mesenteric artery and the three main branches of the coeliac artery, are evident in the illustration (fig. 1), and need no further description.

From the left gastric artery just distal to its origin from the coeliac artery, there arises the very short, common stem of the two inferior phrenic arteries. This stem immediately bifurcates, and the two inferior phrenic arteries pursue their usual course. It should be mentioned here, that the inferior phrenic arteries occasionally arise in a common stem from the coeliac artery, and not rarely from the left gastric artery; but an origin in a common stem from the left gastric artery when this artery is a constituent of a coeliaco-mesenteric trunk, is of considerable rarity (Eaton, '17). In addition to the

inferior phrenic arteries, still another vessel is derived from the coeliaco-mesenteric trunk in this specimen. From the inferior aspect of the coeliac artery, opposite the left gastric artery and just distal to the exit-angle of the superior mesenteric artery, a supernumerary vessel arises. This artery

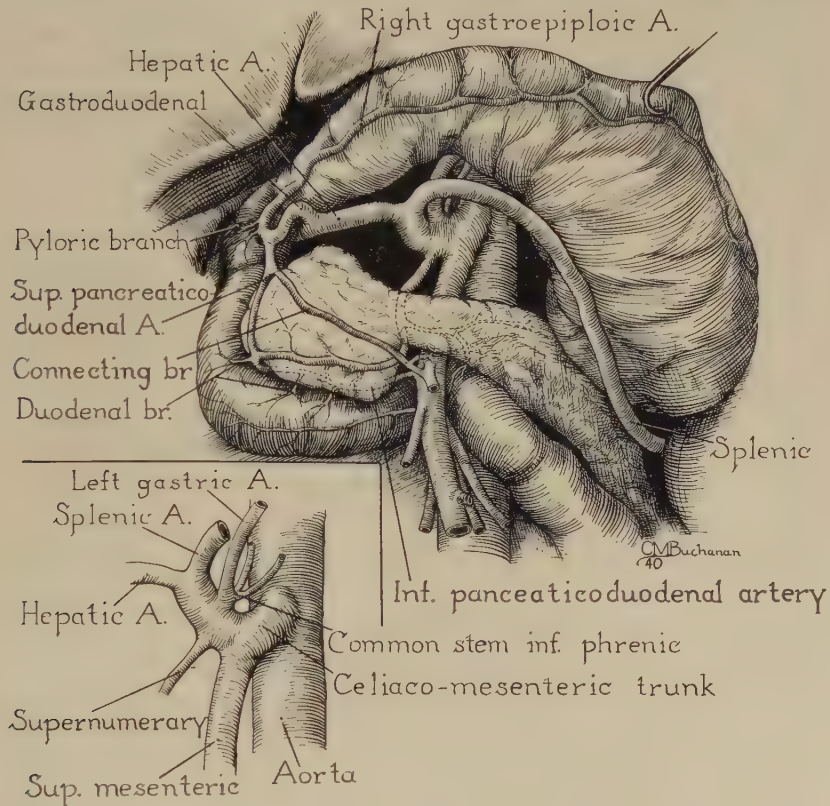


Fig.1 Showing the distribution of arteries over the surfaces of the pancreas and duodenum. The small insert at the lower left shows the coeliaco-mesenteric trunk in detail, and the origin of each of the branches.

passes inferior and posterior to the inferior mesenteric vein and the neck of the pancreas. On the posterior aspect of the neck of the pancreas it gives off a large branch which courses to the left in the substance of the pancreas, supplying numerous vessels to the body and tail of that gland. The main portion

of the supernumerary vessel continues downward, and then curves forward below the lower edge of the neck of the pancreas. Between the right inferior margin of the head of the pancreas, and the junction of the descending and horizontal portions of the duodenum, it anastomoses directly with the superior pancreaticoduodenal artery. At this point, the anastomosis is joined by another artery, the inferior pancreaticoduodenal artery. This latter artery arises from the superior mesenteric artery below the anterior margin of the pancreas, and courses to the right through the substance of the pancreas near the inferior margin of the head of this gland. It supplies branches to the horizontal portion of the duodenum, as well as to the head of the pancreas. The duodenum is further supplied by a moderately large vessel which arises directly from the superior mesenteric artery just distal to the origin of the inferior pancreaticoduodenal artery. This duodenal branch courses directly to the right across the anterior aspect of the horizontal portion of the duodenum, supplying branches to this portion as well as to the lower part of the descending portion of the duodenum.

Still another arterial variation is found in a large connecting branch which arises from the middle colic artery just distal to the origin of the latter artery from the superior mesenteric artery. This connecting branch courses across the anterior surface of the head of the pancreas, and anastomoses directly with the superior pancreaticoduodenal artery near the latter's origin from the gastroduodenal artery. From this connecting branch, just distal to its origin from the middle colic artery, a small branch arises which courses to the left, posterior to the superior mesenteric artery, and, entering the substance of the pancreas, supplies a portion of the body and tail of this organ.

SUMMARY

An unusual coeliaco-mesenteric trunk, and the unique distribution and anastomoses of its branches over the surfaces of the pancreas and duodenum, is described.

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A STUDY OF THE POST-NATAL DEVELOPMENT OF THE SKIN AND HAIR OF THE MOUSE

HELENA F. GIBBS

University of Leeds, England

TWO TEXT FIGURES AND TWO PLATES (EIGHT FIGURES)

INTRODUCTION

A recent study of the development of the skin and the hair in the Australian opossum, *Trichosurus vulpecula* (Gibbs, '38), revealed a close relationship between the thickness of the epidermis and dermis and the development of the different generations of the follicles and their consequent grouping. These features were of evolutionary significance since they confirmed the primitive nature of the Marsupialia in relation to the placental mammals, and at the same time indicated their advance upon the Monotremata. Three phases were noted in the development of the skin, which coincide with the appearance of the first, second and remaining generations of follicles.

The significance of these results suggested the advisability of further investigation by similar methods in order to determine the relationship between the developing skin and hair of other mammals and to explore the possibility of a connection between these phases, should they exist, and the changes occurring in other organs of the young animal.

Very little work has been done on the correlation of the developing skin and hair. Erickson ('31) describes the development of the hair and gives a few measurements of the thickness of the layers of the epidermis which indicate a decrease in thickness. Butcher ('34-'35) shows some relevant figures on the subject, while Loeb and Addison ('11), Loeb ('19-'20) and Thüringer ('24-'25), discuss the function and regeneration of epidermal cells. Loeb and Haven ('29) give quantitative results of epidermis in young mammals.

Phases of hair growth have been described by Dry ('25-'26) and Butcher ('34-'35) but no comparison with the skin has been made. Butcher ('28) shows a correlation between the hair cycles and the development of the ovarian follicles.

MATERIAL AND METHODS

The mouse, *Mus musculus*, being a placental mammal which is naked at birth, was particularly suited for this investigation.

The animals used were bred in the laboratory, fed on a standard diet and kept, as far as possible, under stable conditions. They were killed at different ages so that a series was obtained from the age of 1 to 15 days. After weighing, the animals were chloroformed, and fixed whole in Bouin or 10% formalin.

For histological studies, small pieces of skin were removed from the anterior and posterior dorsal regions and trimmed under the binoculars. For vertical sections the skin was cut with the long sides of the rectangle parallel to the line of the follicles. The samples of skin were embedded in paraffin wax after being left in wax at 56°C. from 6 to 8 hours according to age.

Sections were cut at 8 μ and stained in Weigert's haematoxylin, with picro-indigo-carmin in 90% alcohol as a counter stain. A few transverse sections were stained in Mallory to show the state of the connective tissue.

The thickness of the epidermis and dermis from vertical sections of the anterior and posterior dorsal regions were measured by means of a micrometer eye-piece. Three animals were measured in this way for each day of the first week and thereafter for each alternate day up to the fifteenth day inclusive. These results were calculated in micra and plotted against the age of the animals in days (figs. 1 and 2). Each point on the graph was determined by calculating the arithmetic mean of the grouped series of 120 measurements taken from the three animals of a particular age.

The data were also treated statistically, the standard error, the standard deviation and the coefficient of variation being determined (tables 1 and 2).

OBSERVATIONS

At birth the skin is differentiated into definite epidermal and dermal layers with developing follicles of the primary and secondary generations. No differences due to sex were observed.

Post-natal development of the skin

At birth, the epidermis consists of four well-defined strata. The stratum corneum is a thick layer of completely cornified cells, staining a bright green with picro-indigo-carmin. In transverse section the cell walls show a closely packed honey-comb arrangement. The stratum granulosum is approximately four layers thick and is composed of elongated vertically compressed cells containing keratohyalin granules (Erickson, '31) which stain deeply with picro-indigo-carmin. No nuclei are visible, since keratinisation is in progress, except in the lower cell layers of this stratum where the granules are not so dense. The stratum intermedium contains two or three layers of large irregularly shaped light-staining cells with well-defined oval nuclei. The stratum cylindricum, or basal layer, consists of a well-defined layer of lightly-staining cells with darkly-staining large cuboidal nuclei. Both cells and nuclei appear to be laterally compressed so that their long axes are at right angles to the surface.

At birth the dermis consists of a layer of connective tissue, composed of bundles of collagenous and elastic fibres, the direction of which runs mainly parallel to the surface of the skin. It corresponds in structure to the reticular layer of the dermis, as described by Bloom ('31). The upper layer of the dermis, or capillary layer of Bloom, was not observed in this study, the junction between dermis and epidermis being quite well defined. On the fifth day, grouping of smooth muscle fibres to form arrector muscles, was observed. After the fourth day, connection tissue condenses around the fibre

groups to form connective tissue compartments (fig. 10). The reticular layer passes directly into the stratum subcutaneum or hypodermis, the lower surface of which is irregularly defined by a layer of striated muscle fibres, which stain bright green with picro-indigo-carmin.

The stratum subcutaneum at birth is somewhat similar in structure to the reticular layer, except that it is penetrated by large blood vessels and the collagenous and elastic fibres are more loosely and irregularly arranged. From birth onward, the connective tissue of this stratum is gradually transformed into closely packed fat cells, which at the age of 4 or 5 days form its principal component. As the technique used in this work did not provide for the fixation of fat, the empty fat cells in the sections observed took the form of an open-meshed network as described by Bloom ('31).

The measurement of the thickness of the skin

The epidermis. As the stratum corneum was rarely completely attached to the stratum granulosum, it was found that more accurate results could be obtained by measuring from the upper surface of the well-defined stratum granulosum, to the lower surface of the stratum cylindricum.

The curves obtained by plotting the measurements of the thickness of epidermis in the anterior and posterior dorsal regions against the age of the animal in days, showed three definite phases of identical range (figures and tables 1 and 2).

The first phase extended from 1 to 4 days; the second from 4 to 13 days, and the third from 13 to 15 days. In the first phase the epidermis increased in actual thickness (figs. 3 and 4); and in the second, it decreased in actual thickness by a suppression of the cell layers of the strata granulosum and intermedium (figs. 5 and 6). In the third, decrease in thickness of epidermis ceased, and a comparatively constant condition was maintained, the stratum granulosum being composed of two compressed cell layers and the stratum intermedium of one cell layer, while the cells of the stratum cylindricum were flattened and their nuclei oval in shape.

Throughout the period under observation, the thickness of the epidermis of the posterior dorsal region exceeded that of the anterior dorsal.

The dermis. As stated by Bloom ('31), the boundaries of the reticular layer of the dermis are too ill-defined to permit of its accurate measurement. So far as these measurements are concerned, therefore, the term "dermis" will be used broadly to include the tissue between the lower surface of the stratum cylindricum and the upper surface of the layer of striated muscle. The measurement of the dermis thus defined, however, is somewhat unsatisfactory, owing to the irregularity in the definition of the lower surface of the stratum subcutaneum, and to the effect of varying amounts of fat upon the thickness of this stratum in different individuals. Nevertheless, when the measurements in micra of the anterior and posterior dorsal regions were plotted against the age of the animals in days, three distinct phases were evident (figs. 1-7). In the first phase there was a small rate of increase in the thickness of the dermis which became greater in the second phase, but decreased in the third phase (figs. 3 and 4).

The thickness of the dermis of the anterior region slightly exceeds that of the posterior region throughout the greater part of the curve.

*The post-natal development of the follicle and
its associated structures*

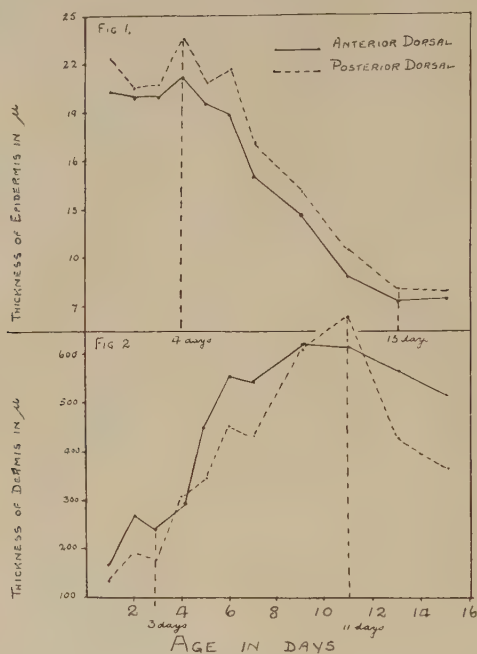
The development of the follicle of *Mus musculus* agrees in general with that of mammals, the formation of follicle layers and the process of keratinisation being similar to that described for *Trichosurus* (Gibbs, '38, p. 615).

So far as this study is concerned no follicle was found to contain more than one fibre. All fibres in the mouse were observed to be strongly medullated.

The sudoriferous or sweat glands. In the early stages of development of the older follicles, a small outgrowth of cells was observed, which appeared to be the rudiments of sudoriferous glands. These soon disappeared, however, and no

sudoriferous glands were observed in connection with any fully developed follicle of the anterior and posterior regions studied.

The sebaceous or fat glands were observed in connection with the larger follicles at 1 or 2 days after birth and from then on appeared at the early "fibre-cone" stage in connection with all later follicles. The young gland is similar in form to



Figures 1 and 2

that described in *Trichosurus* and similar indications of a "hair canal" were found (Gibbs, '38, p. 619). Later, the sebaceous gland takes the form of a small outgrowth on the obtuse angled side of the follicle.

At this stage, the attachment of the arrector muscle, by one of its extremities, to the wall of the follicle just below the gland, and by the other extremity to the lower surface of the epidermis, would suggest that the contracting arrector muscle

TABLES 1 AND 2

Thickness of skin in micra for the anterior and posterior dorsal regions

I. Epidermis					II. Dermis		
DAYS	n.	$a \pm \frac{\sigma}{\sqrt{n}}$	σ	V.	$a \pm \frac{\sigma}{\sqrt{n}}$	σ	V.
Anterior dorsal					Anterior dorsal		
1	120	20.333 $\pm$ 0.274	2.992	14.7	166.000 $\pm$ 3.54	38.80	23.4
2	"	20.066 $\pm$ 0.286	3.149	15.6	264.600 $\pm$ 8.05	88.20	33.4
3	"	20.133 $\pm$ 0.266	2.918	13.7	241.200 $\pm$ 3.46	37.90	15.7
4	"	21.200 $\pm$ 0.342	3.745	17.7	295.200 $\pm$ 4.51	49.37	16.7
5	"	19.733 $\pm$ 0.248	2.720	13.8	450.594 $\pm$ 3.82	41.88	9.3
6	"	19.066 $\pm$ 0.214	2.352	12.3	556.488 $\pm$ 6.65	72.81	13.1
7	"	15.100 $\pm$ 0.228	2.501	16.6	532.584 $\pm$ 3.07	33.69	6.3
8							
9	"	12.700 $\pm$ 0.186	2.044	16.1	612.000 $\pm$ 5.90	64.53	10.5
10							
11	"	9.033 $\pm$ 0.179	1.966	21.8	620.000 $\pm$ 8.86	97.00	15.6
12							
13	"	7.500 $\pm$ 0.160	1.756	23.4	571.950 $\pm$ 5.64	61.74	10.8
14							
15	"	7.667 $\pm$ 0.192	2.104	27.4	512.000 $\pm$ 7.70	84.35	16.5
Posterior dorsal					Posterior dorsal		
1	120	22.533 $\pm$ 0.290	3.181	14.1	137.250 $\pm$ 1.83	20.00	14.7
2	"	20.700 $\pm$ 0.338	3.712	18.0	191.340 $\pm$ 4.28	46.87	24.5
3	"	20.867 $\pm$ 0.318	3.484	16.7	183.600 $\pm$ 2.40	26.24	14.6
4	"	23.734 $\pm$ 0.262	2.862	12.10	305.710 $\pm$ 5.24	57.42	18.8
5	"	21.934 $\pm$ 0.193	2.098	10.0	345.150 $\pm$ 4.43	48.60	14.1
6	"	21.834 $\pm$ 0.240	2.628	12.0	448.650 $\pm$ 3.22	35.30	7.85
7	"	17.365 $\pm$ 0.107	2.1600	6.70	435.450 $\pm$ 4.43	48.50	11.1
8							
9	"	14.266 $\pm$ 0.234	2.568	18.0	697.350 $\pm$ 3.38	37.08	6.1
10							
11	"	10.667 $\pm$ 0.102	1.124	10.50	675.000 $\pm$ 13.05	143.00	21.2
12							
13	"	8.233 $\pm$ 0.190	2.085	25.4	433.800 $\pm$ 4.65	50.94	11.7
14							
15	"	8.100 $\pm$ 0.160	1.748	21.6	371.250 $\pm$ 4.05	44.30	11.9

n = number of measurements.

a = arithmetic mean of each group of 120.

 $\frac{\sigma}{\sqrt{n}}$ = standard error. σ = standard deviation.V = coefficient of variation and $= \frac{100 \sigma}{a}$.

provides the mechanism by which pressure is exerted on the sebaceous gland, thus forcing the fatty secretion into the open follicle (Wildman, '32).

Follicle generations

Vertical and transverse sections of the anterior and posterior dorsal regions from 1 to 15 days, were studied.

The terminology of the follicle generations is based on that adopted in the previous study of *Trichosurus* (Gibbs, '38, p. 622). The following description of follicle generations applies to both the anterior and posterior dorsal regions.

Primary follicles (figs. 3 and 8). At the age of 1 day these first generation follicles, with their accompanying sebaceous glands, are present in a late fibre-cone stage of development. Few, if any, primary follicles are added after birth and their fibres pierce the surface on the second or third day. Their development follows the general plan, but they can always be distinguished from the follicles of succeeding generations by the comparatively greater length and stoutness of the follicle, their thick connective tissue sheaths and the coarse fibres which they produce.

Secondary follicles (figs. 3 and 8). At the age of 1 day, some of these second generation follicles are present in early stages before or just after invagination. They increase in number up to about the third day, the fibres of the majority piercing the surface by about the fourth day. They are far more numerous than the primary follicles, between which they are interspersed in the form of irregular rows. Although eventually their length equals that of the primary follicles, they are always distinguished by the slender form of the follicle, their thinner connective tissue sheath and the type of fibre which they produce.

At the age of 9 days, a lateral flattening of the follicle bulb occurs, which causes the dome of the follicle to become crescent shaped in transverse section. This shape is passed on to the growing fibre which becomes grooved, so that it has concave and convex surfaces, while the medulla is divided

into three columns, the pigment thus being contained in three separate compartments (Salaman, '22).

Tertiary follicles (figs. 4 and 9). The third generation of follicles is first seen on the fourth day as small undifferentiated follicles arranged one on either side of each primary and secondary follicle. By the fifth day, the resulting trio groups each with a primary or secondary as its central follicle, has become general. At this stage, an arrector muscle becomes attached to each follicle. The tertiary follicles follow the general plan of development. They are however shorter and more slender than the follicles previously described.

Quaternary follicles (figs. 5 and 6). The first follicles, comprising the fourth generation, appear on the fifth day, between the tertiary and central follicles of the group to form the five-group stage. They resemble the tertiary follicle in size and form.

Later follicles. On the sixth, seventh and eighth days, new follicles are added as wedge shaped downgrowths between the existing follicles of the groups. These new follicles grow rapidly, become quickly keratinised and soon obliterate the grouping of follicles. They are very numerous, short and slender.

All follicles show rapid growth between the fourth and fourteenth days when their full length has been attained.

DISCUSSION

The mouse, like the more primitive marsupial *Trichosurus vulpecula*, is naked at birth, the follicle and hair development occurring while the young mouse is in the nest. This somewhat exposed post-natal condition provides an environment which may be compared with that of the young opossum in the marsupial pouch.

A comparison of skin and follicle development

Epidermis and dermis (figs. 1 and 2). The three phases indicated by the measurement of the thickness of the epidermis and dermis in the anterior and posterior dorsal regions, do

not exactly coincide, the change in phase of the epidermis following the change in the dermis. Similar phases were described in *Trichosurus*, but in the latter the phases coincided and there was no rapid increase in the dermis during the second phase.

The epidermis and follicle generations. The development of the follicle generations occurs also in three definite phases which agree closely with those found in the epidermis (fig. 1). Consequently while the epidermis increases in thickness (up to the fourth day), primary and secondary follicles develop and their fibres pierce the surface. During the second phase (4 to 13 days), while the epidermis is decreasing in thickness and probably increasing in surface area due to a stretching process, tertiary, quaternary and later follicles appear in rapid succession up to the eighth day. From then until about the thirteenth day all the follicles increase rapidly in length. Lastly in the third phase (from about 13 days), when the epidermis ceases to decrease in thickness, follicle growth ceases and is replaced by increased fibre growth.

So far as hair or fibre growth is concerned, these phases are indicated fairly clearly by Dry ('25-'26) in his description of the coat of the mouse. In the dorsal region he found no new "overhair follicles" (compare primaries and secondaries) after 3 days. This would, therefore, correspond to the first phase when primary and secondary follicles are present. Further, Dry states that the initiation of "underfur follicles" continues up to about 7 days, which again agrees with the initiation of tertiary, quaternary and later follicles during the second phase.

*A comparison of skin and hair development in
Mus musculus and Trichosurus vulpecula*

In comparing these observations with the results of the work on the marsupial, many points of interest emerge.

Only primary follicles are present during the first phase of *Trichosurus*, while secondaries appear and develop during the second phase. Grouping of follicles occurs in the third

phase, when an arrector muscle is added. Moreover, there is an interval between the appearance of each generation of follicles, so that trio- and five-group stages are quite distinct.

Although the same sequence of events appears in the post-natal development of the hair follicles of the mouse, the whole process seems to have been speeded up, and advanced one stage. Thus the secondaries appear in the first phase, while tertiaries and quaternaries and later follicles appear in the second phase—the actual trio- and five-grouping being passed through rapidly. Further, although each follicle is provided with an arrector muscle, as is the case in other placental mammals (Wildman, '32), these muscles are not developed until grouping of the follicles occurs, thus showing a further resemblance to the order of events found in the marsupial. It would seem, therefore, that a recapitulation of the events found in the more primitive marsupial occurs in the post-natal follicle development of the mouse.

The decrease in thickness of the epidermis

The actual decrease in thickness of the epidermis which is characteristic of the second phase of both the mouse and *Trichosurus*, is due for the most part, to the decrease in the number of cell layers and, to a slight extent, to the decrease in thickness of the cells in the strata granulosum and intermedium. This may be due to a period of inactivity in the formation of the epidermal cells—a surprising factor, however, when the rapid activity of the underlying tissues is taken into consideration. Further, a sideways migration of epidermal cells in an attempt to adjust themselves to the rapid growth of the underlying tissues may provide an explanation of this decrease and of the apparent stretching of the epidermal surface. Lateral movement of epidermal cells has been demonstrated by Loeb ('19-'20) in his work on the mechanism of wound healing where he describes the lateral amoeboid movements of the cells toward the wound. Such movement, even if regarded tentatively as responsible for the decrease in thickness of the epidermis, does not explain the

reason for the cessation of a normal increase in the thickness at a time in the life of the young animal, when an increase in the size of all structures would be expected.

Various authors have vaguely indicated such a decrease in other mammals. Erickson ('31) finds, in the caudal region of the rat, the stratum germinativum (i.e. the stratum cylindricum) to be 30 to 40 μ thick at birth, 30 μ thick at 14 days and an average of 20 μ in the adult. Fraser ('28) notes a steady increase in the thickness of the epidermis of the albino rat from the seventeenth foetal to the second post-natal day, while Butcher ('34-'35) starting at the fifth post-natal day, does not actually mention a decrease in the thickness of the epidermis, but his photomicrographs are significant in this respect. His figures of vertical sections of skin from the dorsum of rats show a distinct decrease in the thickness of the epidermis from 5 to 17 days, while the thickness of epidermis at 17 days (compare 13 days in the mouse) is maintained to the adult stage. Jackson and Lowry ('12-'13) state that the integument of the albino rat grows with remarkable rapidity during the first week, after which it decreases in relative weight. This would seem to bear out the observations of other workers on the rat.

Loeb and Haven ('29) in their quantitative studies of the growth of the epidermis in the ear of the guinea-pig, refer to the number of cells in the cell layers and to the proliferative activity at different ages. They comment on the fact that the number of cells and the mitotic activity is definitely lower in the upper layers of the epidermis of the very young and sexually immature guinea-pigs than in the adult, an unexpected result, they suggest, considering that the general growth of these young animals is very rapid. Hence it seems probable that this particular decrease in the thickness of the epidermis is a usual occurrence in mammals.

Loeb and Haven ('29) suggest a phase of inactivity as the explanation of the decrease in the number of cells. It should be noted, however, that in the mouse, this second phase is one of rapid follicle formation and therefore scarcely one of complete epidermal inactivity at least so far as the lower layers

are concerned. This feature of rapid follicle formation itself might be taken as the responsible factor for the decrease in the number of cells in the upper layers, but that a similar decrease occurs in *Trichosurus* when comparatively few follicles are being formed. Also Thüringer ('24-'25) states that all cell layers beneath the stratum granulosum are capable of reproducing new cells. The upper layers should therefore be capable of maintaining the increase in thickness in spite of the rapid follicle formation in the stratum cylindricum. Therefore, the above evidence and the present observations on the mouse suggest that if epidermal inactivity exists, it is apparent more particularly in the cell layers above the stratum cylindricum.

Phases and cycles of development in mammalian organs

It is well known that the growth of hair is cyclic in many mammals, but from the present work it can be seen that the first cycle at least of hair growth, with its marked sub-division into three phases, affects not only the hair but also the skin.

In *Trichosurus* the age at which this first cycle and its three phases were observed could not be stated with certainty (Gibbs, '38, p. 613) but in the mouse the termination of the first phase at the age of 3 or 4 days, of the second at approximately 13 days, and the third at about 15 days, agrees with the data given by Dry ('25-'26) for the hair growth in the mouse.

Butcher ('34-'35), Myers and Myers ('21-'22) and Rice and Jackson ('34) indicate that in the rat the first phase ends by the fifth day, the second by about the seventeenth day, while Butcher notes no further hair follicle growth after the twenty-second day. Further, his figures in plate I show a remarkable resemblance to the condition found in the mouse skin at the critical phase stages. He suggests that the tissues are receptive to a growth stimulus until about the seventeenth day, and also states that in a previous work (Butcher, '28) he found similar conditions in the ovary of the rat. He says that a period of enlargement of ovarian follicles set in about the

sixth day and continued until the seventeenth day, which roughly coincides with the second phase of hair follicle and skin growth. However, he does not regard the gonad as being responsible for this hair growth since the same hair cycles occur in ovariectomized animals.

Engle ('31) dealing with the diameter of the ovarian follicles in the mouse, shows phases comparable, as in the rat, to those described here for the skin and hair. He records a steady increase in the diameter from the fourth to the fourteenth day, after which the growth rate suddenly changes. He states that the increase in follicular diameter appears to be independent of the body weight, while the sudden increase at the fifteenth day follows the appearance at the twelfth or thirteenth day of the first antral cavity filled with follicular fluid. He suggests that an anterior pituitary lobe stimulus releases the follicular hormone at about the thirteenth day causing great follicular growth by the fifteenth day.

In view of this suggestion it is of interest to note that there is a sharp decline in the number of basophil cells in the anterior pituitary of the mouse at about the thirteenth or fourteenth post-natal day (Spaul and Vincent, unpublished).

It would appear, therefore, that there is some relationship between the early post-natal development of the hair and skin and of the ovarian follicles. This is apparently not a case of a direct action of the ovary on the integument, but rather that of some other factor influencing both at the same time.

The controlling influence of the anterior lobe of the pituitary is well known, including its effect on the gonads, while Bissonnette ('35) shows that a marked relationship exists between the hair cycles and the changes in the anterior hypophysis of mature ferrets.

It seems probable, therefore, that a stimulus from some common source exerts a simultaneous controlling influence on the early post-natal development of the ovarian follicles, the skin and the hair.

SUMMARY

1. The sequence of development of the follicle in the mouse from its commencement as a solid downgrowth of cells from the epidermis to the fully formed fibre, agrees with that of mammals generally.

2. No functional sudoriferous glands are developed in the regions observed.

3. The development of the skin and hair follicles from 1 to 15 days is divided into three phases:

First phase. One to 4 days; epidermis increases in thickness; primary fibres pierce the surface; secondary follicles develop. One to 3 days—dermis increases in thickness.

Second phase. Four to 13 days; epidermis decreases in thickness; tertiary and quaternary follicles develop with trio- and five-grouping of follicles; an arrector muscle becomes attached to each follicle; "later" follicles develop and grouping becomes obliterated. Three to 11 days—dermis increases rapidly in thickness.

Third phase. Thirteen to 15 days; decrease in thickness of epidermis ceases; follicle formation and growth ceases. Eleven to 15 days—dermis decreases in thickness.

4. This sequence in the post-natal development of the skin and hair of the mouse (a placental mammal) shows a recapitulation of the process as found in that of the more primitive marsupial *Trichosurus vulpecula*.

5. The three phases of growth appear to be subdivisions of the first cycle of mammalian hair growth reported by various authors.

6. These phases show marked correlation with similar phases in early post-natal development of ovarian follicles, and it is suggested that, in both cases, they are subjected to a stimulus derived from a common source.

ACKNOWLEDGMENTS

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EXPLANATION OF PLATES

All sections (with the exception of fig. 4) were stained in Weigerts' hematoxylin and picro-indigo-carmin in 90% alcohol, and cut at 8μ .

Photomicrographs by J. Manby, University of Leeds.

PLATE 1

EXPLANATION OF FIGURES

3 Vertical section of the skin of a 2 day old mouse, i.e. during first phase, to show primary and secondary follicles before grouping occurs and the thickness of epidermis and dermis. $\times 75$.

4 Vertical section of the skin of a 4 day old mouse, i.e. end of first phase, to show new tertiary follicles and increased thickness of epidermis and dermis. $\times 75$.

5 Vertical section of the skin of a 5 day old mouse, i.e., during second phase, to show decrease in thickness of epidermis and increase in thickness of dermis. $\times 75$.

6 Vertical section of the skin of a 9 day old mouse, i.e., during second phase, to show a further decrease in the thickness of the epidermis and an increase in the thickness of the dermis. $\times 75$.

P.F., primary follicle; S.F., secondary follicle; T.F., tertiary follicle; Q.F., quaternary follicles; Seb.gl., sebaceous gland; Arr.m., arrector muscle.

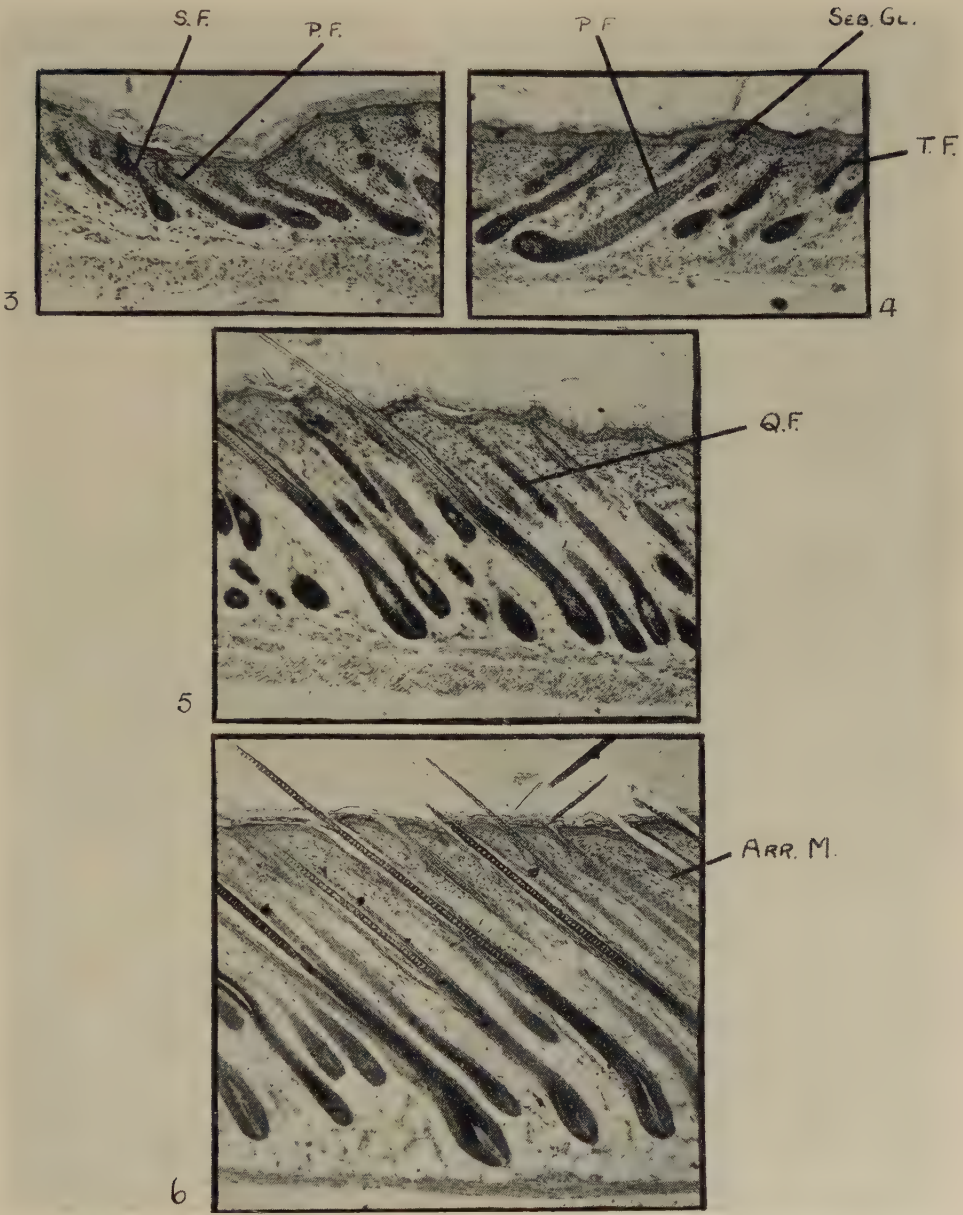


PLATE 2

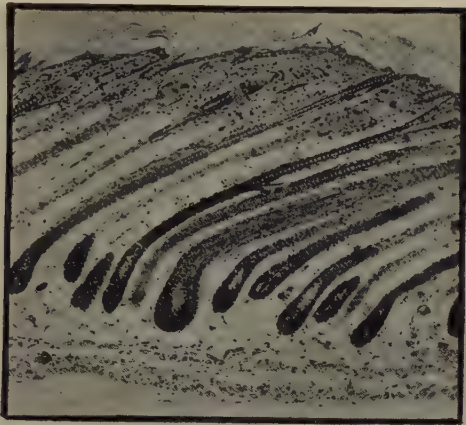
EXPLANATION OF FIGURES

7 Vertical section of the skin of a 13 day old mouse, i.e., during third phase, to show thickness of the epidermis and the dermis. $\times 75$.

8 Transverse section of the skin of 2 day old mouse, i.e., during first phase, to show arrangement of primary and secondary follicles without grouping. $\times 250$.

9 Transverse section of the skin of a 6 day old mouse, i.e., during second phase, to show trio- and five-grouping of follicles. $\times 250$.

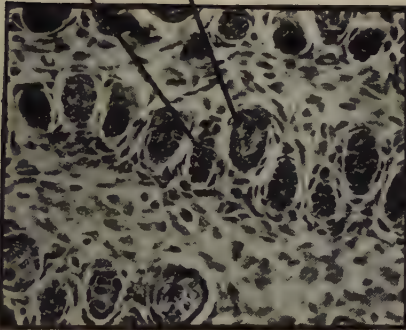
10 Transverse section through skin of 6 day old mouse, i.e., during second phase, to show the arrangement of the connective tissue around the follicle groups. Stained in Mallory. $\times 250$. C.C., connective tissue compartment.



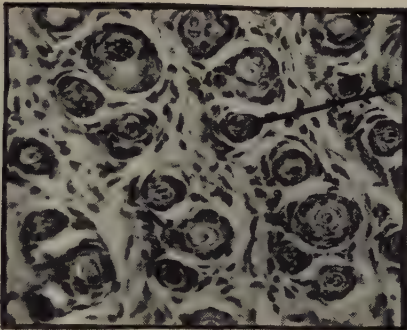
7

S.F.

P.F.

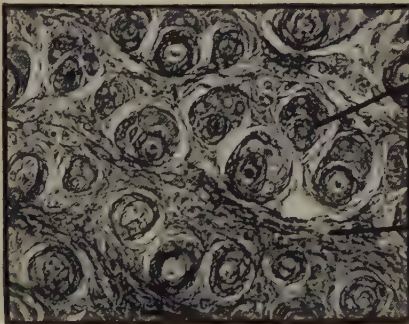


8



T.F.

9



Q.F.

C.C.

10

AUTOPLASTIC GRAFTING OF THE ANTERIOR PITUITARY IN MALE RATS

EUGENE CUTULY

*Department of Anatomy, Wayne University College of Medicine,
Detroit, Michigan*

TWO PLATES (TWELVE FIGURES)

Hypophyseal grafting in the male mammal has not been so extensive as in the female. Hohlweg and Junkman ('32) transplanted pituitary tissue into the kidneys of male rats, but they were interested solely in the histological aspect of the grafts. May ('35) grafted hypophyses from 1-day-old rats to the anterior chamber of the eye of two rats 2 months after hypophysectomy. After a period of 143 days he noted that in both rats the testes had redescended into the scrota and both animals had increased slightly in body weight. Desclin and Grégoire ('36), and Martins ('36 a, '36 b) concerned themselves with the morphological responses of pituitary grafts to estrogen. In 1936 Hill and Gardner reported some effects of intra-testicular hypophyseal transplants in two incompletely hypophysectomized mice in which it was found that the transplants maintained or restored complete testicular function and caused some stimulation of the adrenal cortex. Brief mention was made by Greep ('36) that reproductive function was sustained in male rats by male or female anterior lobe tissue placed in the sella turcica. Some results of autoplasmic grafting in male rats were presented in an abstract by Cutuly ('40). The most complete report to date has been that of Schweizer, Charipper and Kleinberg ('40). These investigators gave details of studies on four completely and six incompletely hypophysectomized male guinea

pigs bearing intraocular homoplastic pituitary grafts. They reported that the transplanted pituitary tissue maintained the tubules and interstitial cells of the testes and the accessory sexual organs, but that it was without effect on the adrenals; the animals increased somewhat in body weight.

Previous studies on hypophyseal grafting (Haterius, Schweizer and Charipper, '35; May, '35; Schweizer, Charipper and Haterius, '37) pointed to the anterior chamber of the eye as a favorable site for transplantation. From a functional standpoint, Greep's ('36) experiments seem to demonstrate that the sella turcica probably is unsurpassed as a location for pituitary transplantation. While the data to be presented confirm this finding, it must be remembered that use of the sella for this purpose is not completely satisfactory, because of possible reestablishment of neural connections. On the basis of maintenance of histological integrity it would appear from the descriptions of Phelps, Ellison and Burch ('40) that intramuscular grafting of pituitary may be quite successful.

In this study the sites chosen for anterior lobe transplantation were the anterior chamber of the eye and the sella turcica of male rats.

MATERIAL AND METHODS

More than fifty male rats were used in this study of autoplasmic grafting of the anterior pituitary gland. Reference to table 1, however, will reveal that data are presented for only six animals with successful ocular grafts, four with successful sellar grafts, six with non-functional grafts and six normal control rats. Data are not included on animals which died, in which tissues were not fixed promptly, and on rats (other than those in table 1) with unsuccessful grafts, despite their value for the determination of the percentage of "takes." "Takes," however, are dependent upon numerous variable factors; for example, it would not seem that much significance can be attached to the percentage of "takes" in this work since minor modifications were introduced in the technique from time to time and because the age of the animals covered a rather wide range.

Grafts were made in the following manner. In the final stage of hypophysectomy the gland was aspirated into a glass tube from which the pieces of gland (usually two halves of the anterior lobe and the posterior lobe) were transferred to a small quantity of normal saline solution. The incision in the neck was quickly closed, and immediately a meridional slit was made through the cornea. Through this slit approximately one-half of the animal's own anterior pituitary gland was inserted into the anterior chamber of the right eye, usually in the direction of the medial canthus. No antiseptics were used or needed in the operation on the eye, but cleanliness was carefully maintained.

At autopsy the whole eye was removed. Its posterior half was cut away and the remaining portion was dropped into Zenker-formol solution. Normal pituitaries were also fixed in Zenker-formol. All other tissues were weighed and then placed in Bouin's fluid. Zenker-fixed material was sectioned at 5μ and stained with Mallory's triple stain; other tissues were cut at 5μ and stained with Harris' or Delafield's hematoxylin and eosin.

The completeness of hypophysectomy was ascertained, wherever possible, by microscopic examination of serial sections of the capsule of the pituitary. Such a course, obviously, was not possible where the ablated gland was replaced in its original location. Except for these latter animals, only rats known to be completely hypophysectomized have been included in this report.

RESULTS

The remarks which follow will serve to amplify the data appearing in table 1. Four groups of animals will be noted: (1) those with successful ocular grafts; (2) those with successful sellar grafts; (3) those with non-functional or briefly functional grafts; data for these animals are exactly what one might expect in hypophysectomized control rats; and (4) normal controls.

TABLE 1

Data on rats with ocular and sellar autoplasmic anterior pituitary grafts

RAT NO.	BODY WEIGHT AT OPERATION	AUTOPSY DATA						OBSERVATION OF EVIDENCE OF GRAFT FUNCTION	HISTOLOGICAL APPEARANCE OF GRAFT
		Body weight	Age of graft	Testes	Seminal vesicles	Adrenals	Thyroid		
		gm.	days	gm.	gm.	gm.	gm.		
Successful ocular grafts									
14	82	92	111	1.72	0.250	0.007	0.006	Few; motile	About 50 days postoperatively
1	149	162	170	2.12	0.255	0.011	Atrophic	Present; motile	Early
3	170	170	170	1.08	0.022	0.010	Atrophic	Few; non-motile	Early period of activity
5	158	145	170	1.22	0.060	0.009	Atrophic	Many; motile.	Early
19	83	150	236	2.19	1.236	0.009	0.011	Many; motile	Early
17	84	130	258	2.36	1.017	0.008	0.009	Many; motile; functional	Early
Successful sellar grafts									
62	148	170	23	2.11	0.457	0.024	0.010	Many; motile;	Many small basophiles, some castration cells, numerous eosinophiles; rich vascularization
61	202	204	97	1.15	0.069	0.022	0.010	rat 28 had	
27	157	204	209	1.90	0.336	0.022	0.015	fertile	
28	154	159	209	2.13	0.674	0.019	0.009	mating	
Non-functional or briefly functional ocular grafts									
64	190	120	29	0.40	Atrophic	0.012	0.009		None
18	95	102	111	0.30	Atrophic	0.013	0.008		Eosinophiles numerous but small; a few small basophiles
Non-functional or briefly functional ocular grafts									
41	80	112	167	0.12	Atrophic	0.007	Atrophic		Graft largely replaced by connective tissue
42	85	82	167	0.12	Atrophic	0.007	Atrophic		Degenerated
43	110	97	167	0.12	Atrophic	0.008	Atrophic		
6	180	168	170	0.34	0.015	0.011	Atrophic	Few; non-motile	Occasional basophiles; no eosinophiles; small capillaries
Normal control values (6 rats)									
Range	88-188	220-500	...	2.32-3.43	0.240-1.155	0.032-0.036	0.016-0.032		Cells undergoing degeneration; small capillaries
Aver.	160	341	...	2.75	0.963	0.035	0.018		

1. Successful ocular grafts. Intraocular transplants of anterior pituitary in this group survived for 111–258 days. Evidence of graft function before autopsy was based upon the appearance of the testes and scrotum. Palpable testes in a turgid scrotum have been demonstrated to represent reliable signs of gonadotropic activity (Cutuly and Cutuly, '38). On this basis two of the six grafts did not “take” immediately. However, results in these two cases did not differ materially from those in the remaining four. Gain in body weight occurred in four animals, one animal lost weight, and in one the weight remained unchanged. Evidently some “growth hormone” can be released by ocular grafts. The testes of all the animals were stimulated (compare figs. 1 and 4 with 3 and 6), but not uniformly as regards tubules and interstitial cells. The degree of tubular stimulation seemed to be proportional to the weight of the testes, while the degree of interstitial cell activation was reflected by the weights of the seminal vesicles. The seminal vesicles of rats 3 and 5 were grossly and histologically atrophic, while the glands of the other four animals were within the normal range. Varying numbers of motile spermatozoa were observed in smear preparations of the epididymides of all rats in this group, with one exception (no. 3). In this latter rat the epididymis contained relatively few, and non-motile, sperm. Special mention should be made of the fact that rat 17 twice had fertile matings and sired normal litters.

None of this group showed evidence of adrenal or thyroid stimulation.

For the most part the intraocular pituitary grafts were small masses of tissue composed chiefly of chromophobic cells (compare figs. 7 and 10 with 9 and 12). Occasional small or large cells with basophilic granulation were noticed. The grafts in rats 17 and 19 contained small eosinophiles in moderate abundance. These latter cells exhibited a narrow border of eosinophilic granules surrounding the nucleus. One or two prominent nucleoli were seen in all types of cells. Vascularization, as a rule, was not rich, which may be a reason

why intraocular transplants of anterior lobe fail to protect hypophysectomized animals completely against the deficiencies consequent on pituitary ablation.

2. Successful sellar grafts. There were four animals in this group and their grafts survived for periods of 23 to 209 days. Two of the rats gained in body weight and two maintained their preoperative weight. There was testicular stimulation in all four animals (compare figs. 2 and 5 with 3 and 6). The tubular stimulation, as in the preceding group, seemed to be proportional to the weights of the testes, while the activation of the interstitial cells was reflected by the weight of the seminal vesicles. In this group of animals, unlike the group with ocular grafts, there was moderate but distinct stimulation of the adrenal glands. As in the former group, however, there was no development of the thyroid. Large numbers of normal spermatozoa were seen in smear preparations of the epididymis of each of the four rats in this group. Rat 28 had fertile matings.

Hypophyseal tissue grafted in the sella appeared largely normal histologically (compare figs. 8 and 11 with 9 and 12). However, basophiles seemed to be relatively more numerous than in normal tissue and small castration cells (fig. 11) were not infrequent. Eosinophiles were not so heavily granulated as in the normal gland, but they were apparently as numerous. Vascularization was quite rich.

3. Non-functional or briefly functional ocular grafts. No significant increase in body weight occurred in any of the animals. Testes, seminal vesicles, adrenals and thyroids in all instances exhibited gross and histological atrophy characteristic of the untreated hypophysectomized rat. A few non-motile spermatozoa were noticed in a smear of epididymis in rat no. 6; all other epididymides were profoundly atrophic with no evidence of any sperm in their tubules. Five of the six grafts displayed signs of gonadotropic activity soon after the transplantation or after an early period of inactivity, but this subsided after a short time.

Grafted tissue was visible histologically in four of the six rats. It will be observed (table 1) that the transplant in rat 64 possessed numerous, though small, eosinophiles, and a few small basophiles. Despite this evidence of granulation, this graft never exhibited signs of activity. Occasional basophiles were found in the grafts of rats 42 and 43. In the remaining three animals the transplants were degenerated or undergoing degeneration.

DISCUSSION

Ocular grafts histologically did not resemble normal anterior pituitary tissue so closely as did sellar transplants, nor was their blood supply so noticeably rich. Possibly a deficient vascularization was responsible for the lack of granulation in the ocular grafts. This might account for the fact that transplants in the sella stimulated the adrenal glands, while those in the eye failed to do so. The inability of either type of graft to activate the thyroid significantly cannot be explained by the data at hand. It is equally difficult to understand how grafts, the cells of which were largely chromophobic, could cause such marked stimulation of the testis.

It should be emphasized that it is not believed that the hormonal output of the grafts was great. In the case of the adrenals and thyroid the absence or low output of adrenal-stimulating and thyroid-stimulating hormones was manifest. The normal appearance of the testes and seminal vesicles of some of the grafted animals, however, might be misleading. The testis would appear to differ from the ovary in that the former seems capable of exhibiting a cumulative response to continuous low levels of gonadotropic. Ovaries of rats subjected to continuous low-level stimulation by gonadotropic hormone, now believed to be a combination of so-called follicle-stimulating and luteinizing hormones, respond by the formation of cystic follicles, thecal luteinization and corpora aberrantia, without ovulation (Witschi and Levine, '34; Cutuly, E., unpublished data).

Whatever the reason for such ovarian behavior, it seems conceivable that a low output of gonadotropin by hypophyseal grafts, together with the inability of the ovary to respond cyclically to a continuous supply of gonadotropic hormone, may help to explain some of the various results obtained in grafting studies on females. Thus, Haterius, Schweizer and Charipper ('35) and Schweizer, Charipper and Haterius ('37) reported continuous vaginal estrus in hypophysectomized guinea pigs bearing intraocular pituitary grafts. Normal cycles were observed in hypophysectomized rats with ocular grafts of anterior pituitary (Martins, '36; May, '37) and in rats with sellar grafts (Greep, '36). Mixed vaginal smears occurred in hypophysectomized rats with intramuscular hypophyseal transplants (Phelps, Ellison and Burch, '40). In a series of hypophysectomized female rats carrying autoplasmic grafts of the anterior pituitary in the anterior chamber of the eye, Cutuly and Haterius (unpublished data) noted occasional instances of continuous vaginal estrus, but in general the vaginal smears were mixed or diestrous.

From the results it seems clear that autoplasmic grafts of the pituitary gland, severed from their normal neural connections, were capable of maintaining the entire reproductive tract of hypophysectomized male rats in a condition anatomically and functionally indistinguishable from the normal. If the idea (Greep, Fevold and Hisaw, '36) is accepted that in the testis tubular stimulation is controlled by a gametokinetic hormone, and interstitial cell stimulation by a luteinizing or interstitial cell-stimulating gonadotropin (Evans et al., '36) it follows that both of these substances were liberated by hypophyseal transplants in the male. Evidence, however, is accumulating which suggests that in the rat the gametogenic and endocrine activities of the gonads may both be controlled by a single gonadotropic hormone (Cutuly, McCullagh and Cutuly, '37; Cutuly and Cutuly, '38; Saunders and Cole, '38; Pencharz, Cole and Goss, '40). Anterior lobe tissue grafted in the eye for a long period of time was able to function as well gonadotropically as sellar grafts, and indeed, as well as the

intact pituitary gland. This would appear to eliminate participation of the nervous system in the release of the gonadotropin(s) responsible for complete testicular activity.

SUMMARY

Successful autoplasmic grafts of the anterior pituitary gland were made in the anterior chamber of the eye of six male rats and in the sella turcica of four male rats. Data are also presented for six male rats in which grafts failed to take or in which function was transient.

Bodily growth occurred in some of the animals bearing functional intraocular or sellar transplants.

Varying degrees of stimulation of the testicular tubular and interstitial elements and of the seminal vesicles of the rats with ocular or sellar transplants were observed. One hypophysectomized rat with an anterior lobe graft in the anterior chamber of the eye had two fertile matings more than 200 days after the transplantation. A rat with part of its own anterior hypophysis grafted in the sella for 209 days also had fertile matings. Such functional evidence, together with the morphological data presented, indicated that successful grafts of anterior hypophysis severed from their normal neural connections, produced one or more gonadotropins necessary for stimulating the gametogenic and endocrine functions of the testis.

Only sellar grafts were adrenotropic, and neither ocular nor sellar grafts caused any detectable activation of the thyroid gland.

Functional grafts in the eye were in general poorly vascularized. The cells of the transplants for the most part were chromophobic, although occasional basophiles and eosinophiles were seen. When present, the basophiles were small or abnormally large, and the eosinophiles were sparsely granulated.

Anterior hypophysis transplanted to the sella appeared more nearly normal histologically than did hypophyseal tissue grafted in the eye. Sellar grafts contained numerous moder-

ately large eosinophiles, many small basophiles and some castration cells. Such grafts were richly vascularized.

The histological aspect of ocular grafts was not necessarily correlated with their functional activity. In some instances grafted hypophyseal tissue found in the eyes of animals exhibiting all of the deficiencies of hypophysectomy was quite similar to that seen in the eyes of rats with functional transplants.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Testis of rat 19 which carried an autoplasic ocular graft of anterior pituitary for 236 days. $\times 70$.
- 2 Testis of rat 27 which carried an autoplasic sellar graft of anterior pituitary for 209 days. $\times 70$.
- 3 Testis of rat 24. Normal control. $\times 70$.
- 4 High power view of figure 1. $\times 277$.
- 5 High power view of figure 2. $\times 277$.
- 6 High power view of figure 3. $\times 277$.

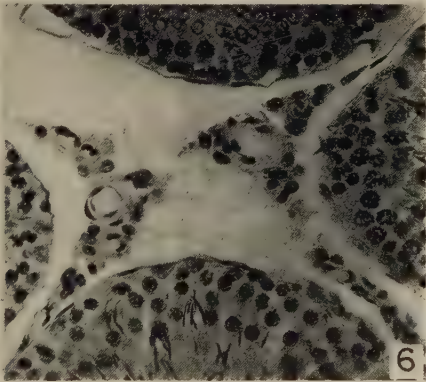
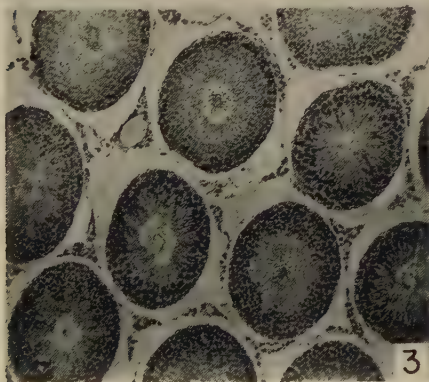
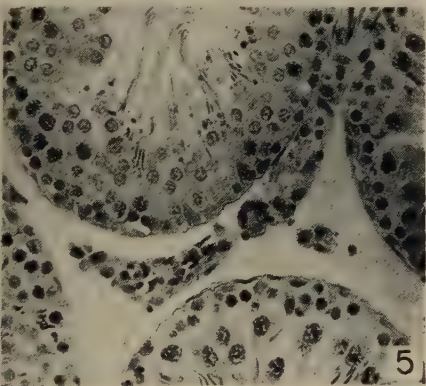
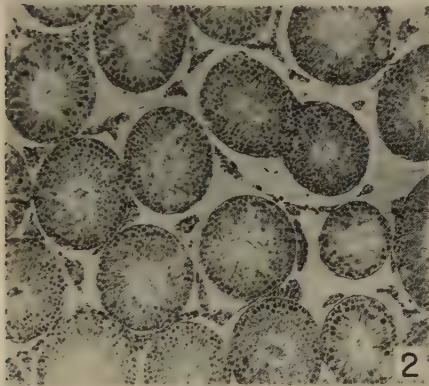
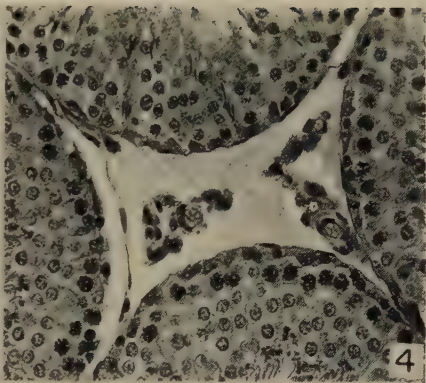
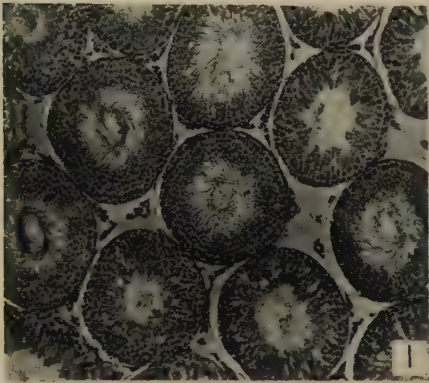


PLATE 2

EXPLANATION OF FIGURES

7 Autoplastic graft of anterior hypophysis in the anterior chamber of eye (rat 19). The graft is seen between the cornea (top of figure) and the heavily pigmented iris. $\times 65$.

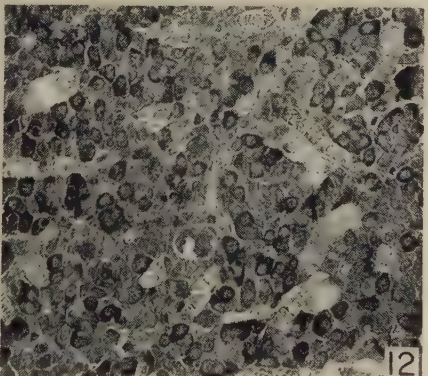
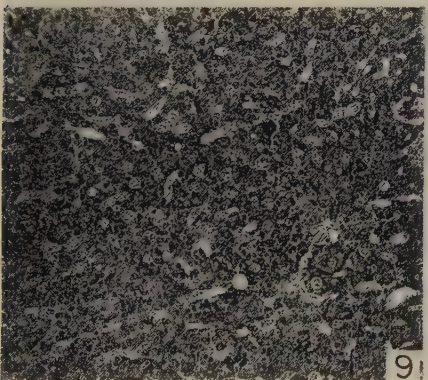
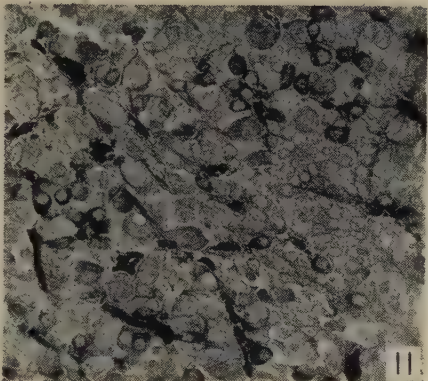
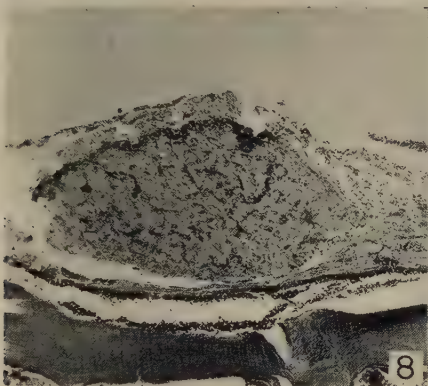
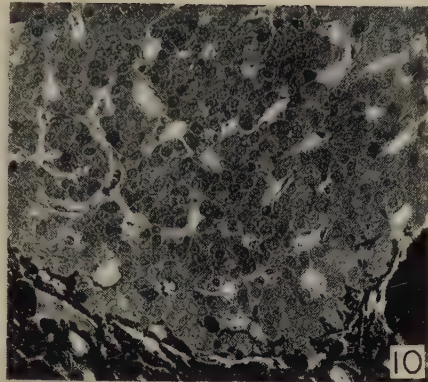
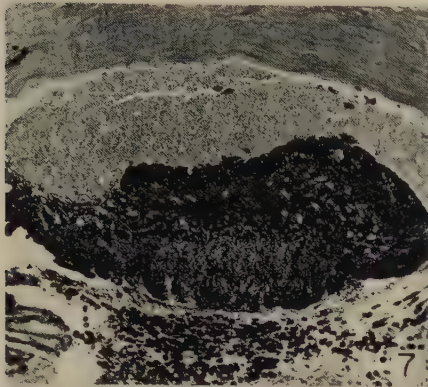
8 Autoplastic graft of anterior pituitary in the sella turcica (rat 27). $\times 65$.

9 Anterior pituitary of normal control rat 24. $\times 65$.

10 Enlarged view of figure 7. Note absence of basophiles; a few sparsely granulated eosinophiles may be discerned (dark border around nucleus); cells are chiefly chromophobic. Vascularization is meager. $\times 137$.

11 Enlarged view of figure 8. Eosinophiles are identified by the dark granules surrounding the nucleus, basophiles by their lightly staining granules and by the macula. Note three vacuolated cells in the upper left quadrant of the figure. Vascularization is rich. $\times 137$.

12 Enlarged view of figure 9. Normal control. $\times 137$.



STUDIES ON THE ENDOCRINES OF REPTILES

II. VARIATIONS IN THE HISTOLOGY OF THE HYPOPHYSIS OF ANOLIS CAROLINENSIS, WITH A NOTE ON THE GOLGI CONFIGURATION IN CELLS OF THE PARS ANTERIOR AND PARS INTERMEDIA ¹

ETHEL G. PORIS

*Department of Biology, Washington Square College of Arts and Science,
New York University*

ONE PLATE (ELEVEN FIGURES)

INTRODUCTION

A vast literature has been built up within recent years dealing with histological and cytological changes in hypophysis, the primary objective being an attempt to correlate such changes with the physiological activities of the organism. Perhaps the greatest interest has been centered on the hypophyseal-gonadal interrelationship, with the greater part of the work limited to the mammalian forms.

As for the reptiles, only one attempt has been made to relate the histology of the pituitary to reproductive activity. Altland ('39) mentions that the basophiles increase in number in the anterior pituitary of *Sceloporus* during the month of May when the animals are at the height of their sexual activity. However, other work on reptiles has involved histological changes in different structures following hormone injections. For example, Turner ('35) injected theelin into adult male skinks (*Eumeces laticeps*) and found a decrease in testis weight and a proliferation of germinal cells. Clapp ('37) injected male *Anolis carolinensis* with theelin and observed an inhibitory effect on the gonads. Evans and Hegre

¹ Accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at New York University.

('38) studied the effects of theelin injections on the histology of the thyroid in the same form and reported an increased activity of the gland. Forbes ('40) implanted pellets of crystalline oestrone in male *Sceloporus* and noted a reduction in volume of the testes, inhibition of spermatogenesis, and the presence of only few spermatozoa. Gorbman ('40) worked on the same form and reported that theelin injections have no significant effects on the thyroid or ovary and that the testes are slightly reduced in size.

In mammals the estrogenic hormones have been shown to affect the gonads indirectly through the pituitary (Moore and Price, '30; Spencer, Gustavson and d'Amour, '31; Leonard, Meyer and Hisaw, '31; Fevold, Hisaw and Greep, '36; Ellison and Burch, '36). It has also been suggested that the thyroid is affected indirectly through the pituitary (Ellison and Burch, '36; Franck, '37; Freudenberger and Clausen, '37; Grumbrecht and Loeser, '38).

In view of these observations it seemed advisable to make a study of the pituitary gland of *Anolis* following the injection of oestrogenic hormone, in an attempt to relate the above changes in thyroids and gonads to pituitary cell types. At the same time it seemed desirable to make some observations on the hypophysis in relation to seasonal variations and following castration.

The purpose of this investigation, therefore, is to study the histological changes in the hypophysis which may be correlated with estrogen injections, castration and season; and, in addition, to describe the Golgi configuration as found in the pituitary of untreated animals.

I wish to acknowledge gratefully the valuable suggestions of Dr. Robert Gaunt and the cooperation of Dr. Harry A. Charipper under whose direction this investigation was carried out.

MATERIAL AND METHODS

The animals used in this study were kept under laboratory conditions as described in a previous paper (Poris and

Charipper, '38). Their diet was further varied, however, by feeding them larvae of *Tribolium confusum*.

To demonstrate the Golgi apparatus, the animals were decapitated, the lower jaw cut away, and the basisphenoid bone and presphenoid cartilage removed to expose the pituitary. The remainder of the skull was then dropped into Champy's fixative (as modified by Severinghaus) for 24 hours. The brain and pituitary were sufficiently hardened by this time to allow them to be easily separated without injury from the surrounding skull bones. The tissue was incubated at 38°C. in 2% osmic acid for 7-10 days, dehydrated and cleared by the dioxan method, and cut in paraffin at 3 μ .

The pituitaries of all experimental animals were fixed in Carnoy-Lebrun and stained with Masson's acid fuchsin and aniline blue as previously described (Poris and Charipper, '38).

Sixteen animals (ten males and six females) were injected intraperitoneally with estradiol-17-propionate (Follacro)² or alpha estradiol benzoate (Progynon-B).³ The first group of animals received daily dosages of 5 R.U. (0.05 cc.) of Follacro for a period of 3 weeks. Many of these animals died and autopsy revealed retention of large quantities of oil in the peritoneal cavity. It was therefore decided that more concentrated material and fewer injections per week would be tried. The second group of animals was injected three times weekly with 20 R.U. (0.01 cc.) of Progynon-B for a period of 4 weeks. Eleven control animals (six males and five females) were injected with equivalent amounts of sesame oil. This experiment was carried out during the months of November, December and January.

For the seasonal study of pituitaries, several animals were killed during each month, starting in July of 1939 and continuing until October of 1940.

Castration was performed under ether anesthesia by means of a single incision in the midventral body wall. Twenty-two

² Kindly supplied by Dr. E. W. Blanchard of Schieffelin & Co.

³ Kindly supplied by Dr. M. Gilbert of Schering Corp.

animals were castrated over a period of 3 months (February, March and April) and were sacrificed at intervals of 3 weeks to $4\frac{1}{2}$ months.

OBSERVATIONS

Oestrogen injections

The most striking reaction to oestrogen injections in the male is the tremendous distension of the blood sinusoids in the anterior lobe of the hypophysis (figs. 3 and 4). This is most obvious in the posterior half of the lobe due to an accumulation of a colloid-like material in the sinusoids of this area. This material stains blue with the Masson technique. In some glands it appears to be perfectly homogeneous while in others it has a very fine reticular structure. Small, clear, spherical vacuoles are sometimes seen along the edges of this material and occasionally blood cells may be found. This blue staining substance rarely extends into the anterior half of the lobe, thus making the contrast between the two regions very marked.

The basophilic area in the posterior ventral part of the gland appears to be reduced in extent over that of the normal gland. Due to the distension of the sinusoids in this region a more conspicuous cord-like arrangement of the basophiles occurs. The cytoplasm in the basophiles stains more faintly than in the normal gland and cell outlines are scarcely visible. The basophiles also appear to be reduced in size as evidenced primarily by the crowding of the nuclei, since the exact cytoplasmic boundaries of the cells are indistinct. The nuclei too are smaller and lose their spherical shape, becoming irregular in outline. In a few of the glands vacuolation of the cells of this area was very prominent. The vacuole forms between the nucleus and the apex of cells bordering on the blood vessels. Occasionally such vacuoles become sufficiently large to push the apical border of the cell wall out into the lumen of the sinusoid (fig. 7). In many pituitaries, regions are observed that are best described as "islands of nuclei."

They appear to be groups of nuclei surrounded by indistinct, vacuolated cytoplasm which gradually grades off into the neighboring colloid-like substance.

The distension of the blood sinusoids is noticeable in the female pituitary also. It appears, however, to be less marked than that observed in the male pituitary. The colloid-like accumulation is apparently less abundant here than in the male gland. The basophiles do not show any of the vacuolation so apparent in some of the male glands. There is, however, as in the males, an apparent reduction in the basophilic area and a blurring of the cell outlines.

No changes in the acidophiles could be noted in either the male or the female pituitaries.

An epithelial cyst was observed in the anterior lobe of one of the injected females. It occupies the entire basophilic area at the posterior end of the lobe. Part of the wall is lined with a cuboidal or a flattened epithelium that forms a distinct membrane, separated from the underlying cells of the hypophysis by a thin strip of connective tissue. The remainder of the wall is made up of normal glandular cells of the anterior lobe. These cells are mostly basophilic elements which normally occur in this region. However, along the lateral walls of the cyst a few acidophilic cells may be seen. In restricted areas of the wall long cilia project from the cells into the lumen. They are for the most part associated with the cuboidal epithelium. In a few sections the pituitary cells forming the cystic lining appear to be disintegrating. Cell outlines are not visible and the nuclei are directly exposed to a mass of small blue-staining spheres along the margin of the cyst lumen.

In the cavity of the cyst there is a large spherical coagulum which stains deep blue. Concentric lines give the impression that this structure has grown by accretion. It is separated from the cystic lining by a fairly large space in which are scattered patches of a lighter blue material containing small deep blue spheres. Some of these masses have actually reached the surface of the central sphere and seem to be con-

tributing to its increase in diameter. Numerous nondescript red-staining areas are scattered throughout the central coagulum. These are probably blood elements and nuclei of degenerated cells.

Thyroids. The thyroids of the injected animals exhibit greater histological activity than those of the controls. This activity is manifested by an increase in the height of the follicular epithelium as well as greater vacuolation of the margins of the colloid. The female thyroids tend to show a slightly greater colloidal vacuolation than the male glands.

Gonads. The histological picture of the testes of the injected males gives indication of reduced activity as compared with the testes of normal control males killed at the same time of year. Control testes show all stages of spermatogenesis, including completely formed sperm. The latter, however, when present, are small in number. In the injected males there are fewer secondary spermatocytes, and an almost complete absence of spermatids and spermatozoa. The testis of one animal, which shows marked changes in the pituitary, also exhibits the most complete inhibition of the gonad. No mitoses are visible in this gonad and only resting spermatogonia with a few primary spermatocytes are present along the edges of the tubules. The central portions of the lumina are filled with a loose reticular material in the interstices of which cells occasionally occur.

The ovaries of the injected females presented no detectable histological variations which could be attributed to the injections.

Castration

Histological study of the pituitaries of animals that had been castrated as long as 10½ weeks revealed no detectable differences from those of normal controls. Cell counts were therefore made, the results of which are indicated in table 1. Of the castrates counted, four had been castrated 7 weeks, one 8 weeks, and one 10½ weeks. The only statistically significant

change noted is a slight increase in percentage of basophiles in the anterior lobes of the castrate animals.

Three animals that had been castrated for $4\frac{1}{2}$ months were the only ones that showed any obvious microscopic alterations in the cells. The basophilic cells in the posterior region of the gland differed from those of normal glands by the presence, in most of the cells, of a small homogeneous acidophilic mass near the nucleus (fig. 5). It generally has the appearance of an irregularly shaped solid mass, but occasionally it resembles a thick ring with a clear central area. Very few of the basophiles bordering on the blood sinusoids in the anterior region of the gland, as well as a few of the chromophobes, show similar structures.

TABLE 1

Differential cell counts in the anterior lobe of castrate and normal lizards

CELL TYPE	MEAN	S.E. _m	M ₁ -M ₂	S.E. _d	DIFF. S.E. _d	NUMBER OF ANIMALS
Anterior acidophiles						
Control	22.1	0.402	-2.2	0.988	2.22	5
Castrate	19.9	0.903				6
Posterior acidophiles						
Control	16.02	0.676	-1.26	2.044	0.61	5
Castrate	14.76	1.93				6
Basophiles						
Control	32.3	0.906	+4.2	1.05	4.00	5
Castrate	36.5	0.531				6

Seasonal changes

The description of seasonal changes will be divided, for convenience, into three phases, namely, spring, summer and fall, and winter. In every instance the changes as described here occur in the anterior region of the pars anterior, no changes being noted in the intermediate lobe.

A. Spring. The spring pituitary is typically characterized by a very weak acidophilia (fig. 10). The eosinophiles border-

ing on the blood sinusoids contain a finely granular cytoplasm which stains light pink. These cells vary in height somewhat but the majority of them tend to be rather low. The granules accumulate only in that area of the cells between the nucleus and the blood sinusoid. A few rounded cells staining faintly acidophilic may be seen between the cell cords.

Many cells bordering on the blood sinusoids are very tall and filled with fine faintly staining basophilic granules. Sometimes one entire side of a sinusoid may be lined with these cells alone. At other times they are scattered in groups between the acidophiles. Most of the cells between the cell cords are similar in their staining reaction to the basophiles.

B. Summer and fall. In most of the summer glands, and especially those of animals killed in August, there is a definite increase in the staining capacity of the acidophiles as well as an apparent increase in the number of these cells both on the blood sinusoids and between the cell cords (fig. 9). The cells are definitely larger and give the impression of being swollen with secretion material. The granules are no longer fine but seem to have fused to form tightly packed clumps or even a solid eosinophilic mass. The nucleus is often compressed to one end of the cell so that it is crescent shaped. The acidophiles between the cell cords also appear to be present in greater numbers and stain more intensely than those of the spring glands. Only a few basophiles could be detected scattered among the acidophiles on the sinusoids.

The cells between the cell cords, with the exception of the acidophiles, show a narrow rim of cytoplasm which is, for the most part, devoid of granules. They show a basophilic reticulum and some scattered basophilic clumps.

At the beginning of July some glands showed the same apparent increase in number of acidophiles as described above but they stained less intensely, thus resembling more closely the cells of the spring glands. Two female glands of this period also showed a large number of basophiles.

C. Winter. The acidophiles during the early part of this period are still intensely staining and numerous but are lower

than the previous ones. The basophiles and chromophobes are essentially similar to those of the previous period.

Some of the glands fixed in January showed a decrease in number of acidophiles and a reduction in their staining capacity. These cells are also extremely low. The remaining cells lining the sinusoids are much taller than the acidophiles and are filled with fine basophilic granules. The greater proportion of cells between the cell cords show a faintly basophilic cytoplasm.

Golgi apparatus

Intermediate lobe. The Golgi apparatus in the intermediate lobe is an elaborate structure that generally takes the form of a fine network (fig. 8). In carefully bleached slides the network is seen to consist of anastomosing strands of osmiophilic material. It is a fairly large structure and may occupy from one-fourth to one-half the length of the cell. In practically all cases the apparatus was observed to lie proximal to the nucleus in that portion of the cell bordering on the blood capillary. The distal end of the apparatus lies close to the nucleus and at times appears to cap its base. In some cells the Golgi apparatus could be seen to extend to one side of the nucleus, in which cases it has a narrow strand-like appearance. In no instance, however, was the configuration found to lie between the nucleus and the base of the cell.

Anterior lobe. The Golgi apparatus in the acidophiles of the anterior lobe (fig. 11) is a compact structure made up of heavy, closely-woven osmiophilic threads. Sometimes the strands are arranged in the form of a ring enclosing a clear, central area. There is no uniformity in orientation of this apparatus in the cell. It may be found occupying a region in the cell to one side of the nucleus or between the nucleus and the blood sinuses. The apparatus is in close apposition to the nucleus and at times appears to cap it in a fashion similar to that described for the intermediate lobe cells.

In the basophiles the Golgi apparatus is not a compact structure as it is in the acidophiles (fig. 11). Instead it has an

elongate strand-like appearance and lies in the cytoplasm a little to one side of the nucleus. The threads may present slight thickenings or varicosities which give the impression of a string of beads. In many cells it is not a continuous structure but is made up of fragments of osmiophilic material. These fragments may vary from granules to small threads.

In between the cell cords are other cells with scanty cytoplasm which have been interpreted as chromophobic elements. In these cells two somewhat different arrangements of Golgi material may be observed. The one most frequently seen is a small compact structure which appears solid in most of the cells. In some, however, osmiophilic strands can be detected forming a dense net similar to that of the acidophiles. Another type that is present, but less frequently than the first, is a narrow strand similar to that seen in some of the basophiles. Unlike that of the basophiles, however, it is not fragmented and is as a whole more heavily impregnated.

DISCUSSION

Oestrogen injections

The distension of blood sinusoids in *Anolis* pituitaries following oestrogen injections is not an unusual phenomenon. Many investigators have reported vascular changes in the hypophyses of mammalian forms under similar conditions. Wolfe ('35) observed in the hypophysis of oestrin-treated rats that the capillaries in many regions were extremely dilated, emphasizing the cord-like arrangement of the anterior lobe cells. Halpern and d'Amour ('34 and '36) noted a marked hyperaemia and an apparent increase in the blood vessels in oestrin-treated rats. Bacsich and Folley ('39) also report marked hyperaemia and numerous hemorrhages in the pituitaries of female rats treated with oestradiol monobenzoate. Cramer and Horning ('36) found that the pituitaries of three mice whose skins were painted with oestrin were deeply congested and hemorrhagic.

The presence of colloid in the blood sinusoids of the pituitary following oestrogen treatment, as described herein for *Anolis*, is apparently unusual. Although colloid has been observed in the anterior pituitaries of mammals following oestrogen treatment, it has rarely been seen in the blood capillaries. Wolfe, Ellison and Rosenfeld ('34) describe colloid in the residual cleft of most female rats injected with pregnancy urine. It was also found occasionally in different phases of the oestrus cycle but never in such high percentage. Desclin ('35) states that the hypophyseal cleft of oestrin injected rats is regularly filled with colloid. Wolfe and Wright ('38) noted large focal masses of colloid between the anterior lobe cells of female rats injected with oestrin for 80 days. Kirkman ('37) studied the anterior hypophysis of the guinea pig during the oestrus cycle and reports that small traces of colloid may be found at any stage of the cycle but that there is a slight increase during oestrus.

The presence of colloid in the blood sinusoids of *Anolis* suggests an excessive secretion of the basophiles into the vascular channels of the hypophysis. This seems reasonable in view of the fact that the accumulation is greatest in the basophilic area of the pituitary and rarely extends into the anterior portion of the lobe. Rioch ('38) states, concerning the pars anterior of normal mammals, that "the anatomical evidence with regard to the presence of 'colloid' in the vessels is in favor of the theory of direct secretion into the blood. It must be regarded only as circumstantial evidence, however, as the chemical relationship of the 'colloid' to the hormones is unknown."

The decreased staining capacity and the vacuolation of basophiles in this area of the anterior lobe are interpreted as indications of degranulation and increased secretory activity. These histological changes have been observed repeatedly in mammals by numerous investigators. Severinghaus ('37 and '38) in his reviews reports that in general there is a degranulation of basophiles accompanied by an enlargement of the Golgi apparatus and an increase in size and number of

the mitochondria. He believes that the gland is manufacturing and pouring out its secretion at an enormous rate.

Vacuolation of the basophiles also has been described in mammals. Wolfe and Wright ('38) brought about a vacuolation of anterior lobe cells in rats by prolonged injection of oestrin. They believe it to be degenerative in character. Schmidt and Starkey ('38) report that mice injected with oestrin for 9-12 days show degenerative changes in the pituitary as determined by granular loss and vacuolation in practically all cells. The vacuolation of the basophiles observed in the present work is also regarded as a degenerative phenomenon following over-stimulation by oestrogen injections, especially so in those pituitaries that show necrotic masses of cells, described here as "islands of nuclei."

The greater response of the pituitary of the male animal to oestrin injections as compared to the response of that gland in the female to similar treatment, is somewhat puzzling. In general, it has been shown for mammals that a greater amount of female hormone is required to bring about changes in the male pituitary comparable to those in the female pituitary. Schmidt and Starkey ('38), however, also found that the anterior pituitary glands of oestrin-treated male mice showed more striking changes than the female glands. Zondek ('36) reports that prolonged treatment of rats with follicular hormone results in enlargement of the male pituitary while the female pituitary is macroscopically unaltered.

In 1930 Meyer, Leonard, Hisaw and Martin announced the important fact that oestrogen administration lowered the gonad-stimulating potency of the rat pituitary. Later Fevold, Hisaw and Greep ('36) suggested that excessive stimulation of the pituitary by oestrin results in secretory exhaustion and consequent partial involution of the gonad. Clapp ('37) showed that theelin injections in male *Anolis* resulted in gonad atrophy. The same observations are reported in this paper. If the colloid in the pituitary of *Anolis* is a product of secretion, and if it represents gonadotropic hormone, one should expect an increase in gonad-stimulating potency. This

is not in harmony, however, with the mass of physiological evidence obtained in mammalian forms and would hardly explain the inhibition of testis activity. It may be possible that over-stimulation results in the production of faulty hormone, so that even though large quantities are being released it is less potent than hormone that is produced under normal conditions.

Another possibility that cannot be overlooked is that the colloid present in the blood vessels may represent excessive secretion of thyrotropic hormone. Evans and Hegre ('38) reported that thyroids of ovulating female *Anolis* were more active than those of other females or males. Marza and Blinov ('36) found that the thyroids of pigeons also appeared histologically more active at times of sexual activity and ovulation. Evans and Hegre injected *Anolis* with theelin during the winter months and stimulated the thyroids of males and females to a marked degree. In mammals, the effects of theelin injections on the thyroid vary. Some investigators report no change (Montpellier and Chiapponi, '30; Shumacker and Lamont, '35); others report histological signs of inactivity (Franck, '37; Grumbrecht and Loeser, '38, and Bacsich and Folley, '39); while a few investigators report increased activity of the thyroids (Freudenberger and Clausen, '37; Pincus and Werthessen, '33, and Amilibia, Mendizabal and Botella-Llusia, '36). In the latter cases it is believed to be due to a stimulating effect on the anterior pituitary. Amilibia, et al., suggest that the contradictory results obtained are due to a difference in length of time of injections. Hertz and Kranes ('34) showed that longer injections of anterior pituitary extracts into rabbits brings about involution of the thyroid while shorter periods of injection result in thyroid hyperplasia. Pincus and Werthessen ('33) found that the increased weight of the thyroid is at a maximum after 5 days of oestrin injection and decreases regularly to a minimum after 40 days' injection. Theelin injections in *Anolis* continued for a period of 1 month in the present investigation, however, does not alter the initial increased activity of the thyroid as observed by

Evans and Hegre following injections for a shorter period of time (18 days).

The action of theelin on the thyroid in *Anolis* is probably one through the pituitary gland as has been indicated in mammals. Further experimentation would be necessary to determine whether or not the colloid in the pituitary of *Anolis* is in any way related to an increased production of thyrotropic hormone.

The epithelial cyst described in the pituitary of one of the injected females is not necessarily related to secretion or to the oestrogen injections. It is included here merely as of confirmatory interest in relation to an observation made by Altland ('39). In his paper he reports observing a ciliated cyst in the anterior lobe of the pituitary of the fence lizard (*Sceloporus undulatus*). He found no indication that this cyst was involved in the secretory process. Epithelial cysts in the pars anterior of the pituitary have also been described in other animals (guinea pig: Vanderburgh, '17; rat and *Didelphys*: Martins, '33; domestic fowl: Collin, '26; human: Rasmussen, '28; and rat: Oppen, '40). The cyst described in the present work may be regarded as an embryological rest originating from Rathke's pouch. This interpretation is most accepted at present for other forms in which such structures are found.

Castration

With the exception of a few reports (Fichera, '05; Zahl, '37, and Payne, '40) all studies concerned with castration effects on the pituitary have been limited to mammalian forms. The castration cells so characteristic of rats have not been observed in any of the lower vertebrates studied; in fact, Payne ('40) found, in the chick, that there was a disappearance of vacuolated basophiles in the castrate animal. The increase in number of basophiles in the castrate pituitary of *Anolis* is consistent with observations on pituitaries of other forms. This observation tends to lend support to the mass of evidence

associating the basophiles with the production of gonad-stimulating hormone.

The significance of the eosinophilic staining structure, present primarily in the basophiles of animals castrated for a period of $4\frac{1}{2}$ months is difficult to ascertain. It may represent stored secretion material which has undergone some chemical change and therefore stains acidophilic, although present in a basophilic cell.

Seasonal changes

The acidophiles apparently pass through a secretory cycle which may be interpreted as follows: 'In the late summer and fall the intensely staining acidophiles represent cells which are producing and accumulating large amounts of secretion material. In the early winter glands the acidophiles still are intensely staining but are lower than the previous ones. This decreased height of the cells may be construed as evidence of granular loss. Altland ('39) pointed out the difficulties many investigators encountered in attempting to trace stages in degranulation of the acidophiles. He noted in *Scleroporus* that there were no cytoplasmic alterations to give evidence of granular excretion. He found no acidophiles with scattered granules and suggests that the acidophiles decrease in length as soon as the granules dissolve. This is not entirely true of *Anolis*. Besides the difference in the length of the cells, there is a definite decrease in staining capacity of the acidophiles as well as a less compact arrangement of the granules in these cells. The latter, which begin to make their appearance in January, but which are more characteristic of the spring pituitaries, are probably a further step in degranulation.

The apparent increased basophilia during the spring and early summer is consistent with the observations of Altland ('39) on the fence lizard, and also agrees with the numerous studies on seasonal breeding forms in other classes of vertebrates.

It is not possible to state the exact relation that these changes bear to the reproductive cycle. At the time when the gonads are most active the acidophiles are in various stages of degranulation and there is an apparent increase in the number of faintly staining basophiles. An assay of the gonadotropic potencies of pituitaries from different seasons of the year might throw further light on this phase of the problem.

Golgi apparatus

In view of our present concept of the role played by the Golgi apparatus in secretion, the presence of an elaborate structure in the cells of the intermediate lobe strengthens the previous suggestion that this lobe is a highly active one (Poris and Charipper, '38; Kleinholz, '38). Altland ('39) also noted an elaborate structure in the intermediate lobe cells of the fence lizard. In this form, the Golgi apparatus caps the nucleus in the region of the cell distal to the capillary. This is contrary to what was observed for *Anolis*, in which the apparatus lies between the nucleus and the capillary border but also occasionally may be seen to extend along the side of the nucleus.

In *Anolis* two types of Golgi configurations were observed in the chromophiles of the anterior lobe. The presence of two types of Golgi figures has also been reported in mammals by Addison ('16), Atwell ('32), and Severinghaus ('33). Among the lower vertebrates, Levenstein ('39) reported a similar condition in the transitional lobe of the goldfish pituitary, and Siler ('36) in the anterior lobe of the garter snake. The two types of Golgi apparatus in the chromophobes of *Anolis* show a close similarity to those present in the chromophiles. Siler and Levenstein find similar conditions to exist in the garter snake and goldfish pituitaries, respectively. Severinghaus ('33) also described two types of chromophobes in mammals and suggested that the acidophiles arise from one type and the basophiles from the other type. This same relationship may be present in *Anolis*.

SUMMARY

1. Oestrogens injected into *Anolis* for a maximum period of 1 month produce a distension of blood sinusoids in the anterior lobe and an accumulation in these sinusoids of a colloid-like material. The latter may represent gonadotropic or thyrotropic hormone.

2. The basophiles in the posterior region of the anterior lobe of injected animals appear smaller, stain more faintly, and have less definite cell outlines than those of normal control glands.

3. In some injected males vacuolation of the basophiles is conspicuous and is interpreted as being a degenerative change due to overstimulation.

4. The thyroids of the injected animals are more active histologically than those of normal controls, indicating the possible release of thyrotropic hormone from the pituitary.

5. The testes of injected males show signs of inhibition of spermatogenesis. This is probably due to an indirect action through the pituitary. The ovaries of injected females show no histological alterations.

6. The pituitaries of animals that had been castrated for a period of 7 to 10½ weeks show no obvious histological changes but cell counts reveal a slight increase in percentage of basophiles. The basophiles of the anterior lobe of 4½-month castrates contain an irregular acidophilic mass near the nucleus.

7. Seasonal changes are described in the anterior lobe. In general, the pituitary is deeply acidophilic in late summer, fall and winter, and faintly acidophilic in the spring.

8. The Golgi apparatus in the intermediate lobe is an elaborate network and is more or less similar in all cells. The two types of configurations described for the chromophobes of the anterior lobe may be related to the two varieties present in the chromophile cells.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 High power magnification of the region of the anterior lobe outlined in figure 2. The dark cells are acidophiles, the light ones basophiles. The spaces are blood sinusoids. $\times 250$. Carnoy-Lebrun. Masson stain.

2 Median sagittal section of the pituitary gland of a normal male. Anterior end is to the left. $\times 60$. Carnoy-Lebrun. Masson stain.

3 Median sagittal section of the pituitary gland of a male injected with oestrin. Note the contrast between the posterior and anterior portions of the anterior lobe. $\times 60$. Carnoy-Lebrun. Masson stain.

4 Higher magnification of posterior region of the anterior lobe outlined in figure 3. Note the enlarged sinusoids filled with a colloid-like substance. $\times 250$. Carnoy-Lebrun. Masson stain.

5 Basophilic area of the anterior lobe of a male castrated for $4\frac{1}{2}$ months. Note the dark acidophilic areas in the cells. $\times 1200$. Carnoy-Lebrun. Masson stain.

6 Basophilic area of the anterior lobe of a normal male. $\times 1200$. Carnoy-Lebrun. Masson stain.

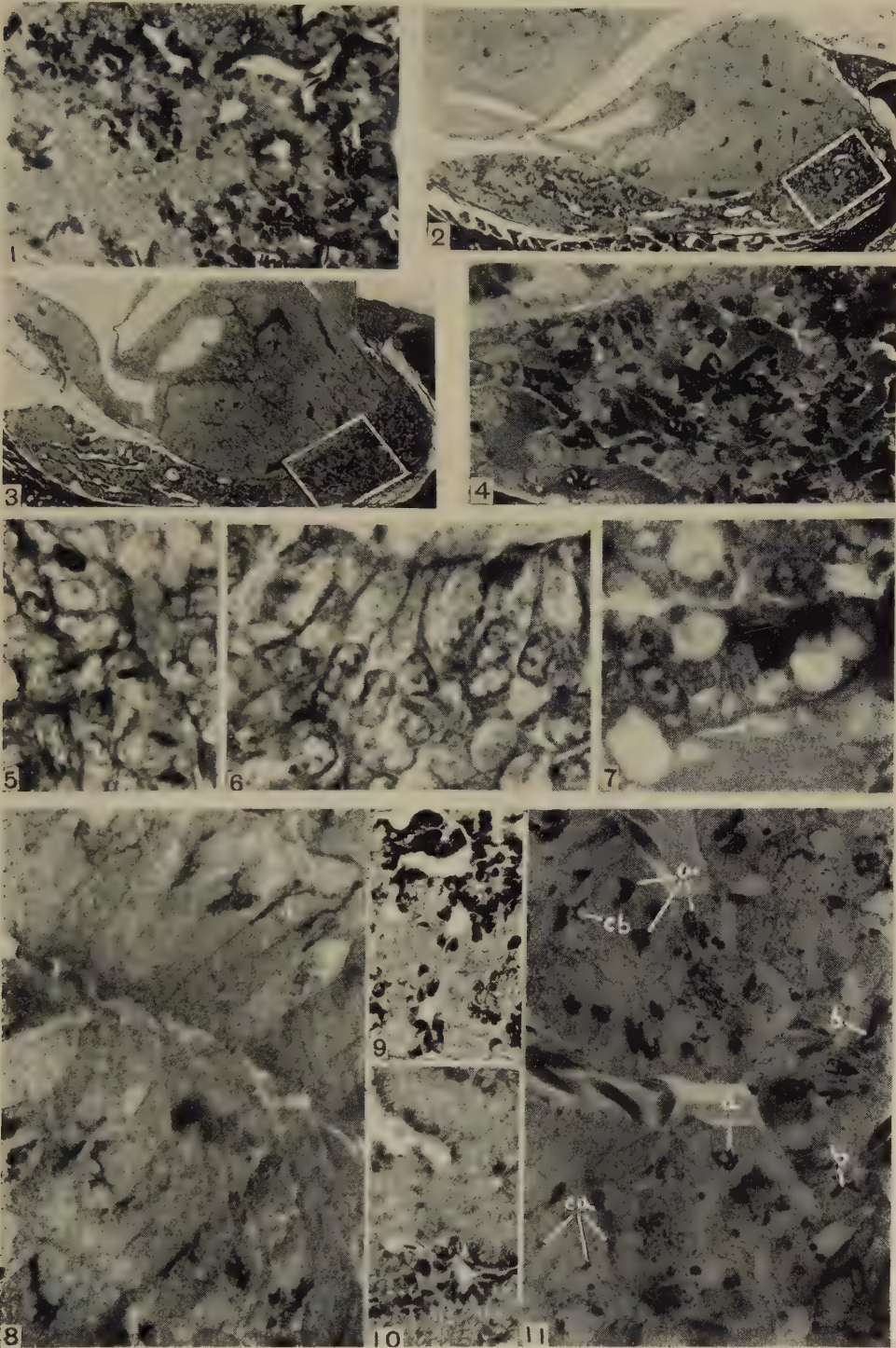
7 Basophilic area of the anterior lobe of a male injected with oestrogens. Note the large blood sinusoid in the lower part of the photograph. The clear areas in the cells are vacuoles. $\times 1200$. Carnoy-Lebrun. Masson stain.

8 Golgi configurations in the cells of the intermediate lobe. Two rows of cells are indicated, one on each side of a blood capillary. The nuclei appear light and lie at the bases of the cells. $\times 1200$. Modified Champy.

9 Portion of the pars anterior of an animal killed in the fall. Note the dark acidophiles filled with secretion. $\times 250$. Carnoy-Lebrun. Masson stain.

10 Area similar to that shown in figure 9 but from an animal killed in the spring. Note the pale acidophiles lining the sinusoids. $\times 250$. Carnoy-Lebrun.

11 Golgi configurations in the anterior lobe. a, Golgi apparatus in acidophiles bordering on a blood sinusoid; b, thread-like Golgi in the basophiles; ca, small compact Golgi of chromophobes; cb, strand-like Golgi of chromophobes. $\times 1200$. Modified Champy.



CHANGES IN THE CONTENT OF IODINE COMPOUNDS AND IN THE HISTOLOGICAL STRUCTURE OF THE THYROID GLAND OF THE PIG DURING FETAL LIFE

ROBERT M. RANKIN¹

Department of Anatomy, Johns Hopkins University, Baltimore, Maryland

TWO CHARTS AND ONE PLATE (FOUR FIGURES)

The purpose of the observations here presented was to follow the development of function of the thyroid gland during intra-uterine life by a quantitative determination of its iodine-containing compounds; and to investigate changes in its histological structure which might be correlated with changes in the chemistry of the gland. Since thyroxine, or a protein containing thyroxine, is the active principle of the thyroid, the demonstration of this substance in the gland may be considered a logical test of its function. Recent improvements in the micro-determination of iodine make such a demonstration possible. Consequently, in this study, the quantity of thyroxine iodine, and in addition, the quantities of total iodine, di-iodotyrosine iodine, and inorganic iodine have been determined in a series of glands from fetal pigs.

MATERIAL AND METHODS

The fetal pigs were collected under conditions described elsewhere (Flexner and Gersh, '37). The fetal thyroids were usually removed within 2 hours after the death of the mother; occasionally the time interval was 4 hours. The glands were weighed and then dried to constant weight in an oven at 75° to 80°C. After drying they were pulverized and stored in a

¹ Henry Strong Denison Scholar for 1940-1941.

desiccator at room temperature until used. It was found that there was no change in the thyroxine content after prolonged storage (2 months).

The thyroxine content was determined according to the micro-method outlined by Palmer, Leland and Gutman ('38) which utilizes the method of iodine determination of Trevor-row and Fashena ('35). From 2 to 150 mg. (depending on the thyroxine content) of the powdered glands were hydrolyzed in 2 N NaOH over a 2 N H_2SO_4 bath for 16–18 hours. The thyroxine was then extracted with n-butyl alcohol and the extract evaporated to dryness. The ash was dissolved in water and the iodine liberated by heating to 195°C . in a digestion mixture of H_2SO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$ and CeSO_4 . Phosphorus acid was added and the mixture steam-distilled in a closed system through which a stream of air was blown. The iodine was collected in 0.1 N Na_2CO_3 ; this solution was evaporated to about 10 cc., acidified, bromine vapor added, and then further evaporated to 5 cc. After adding potassium iodide and starch the solution was titrated against 0.001 N sodium thiosulfate.

For the measurement of the total iodine, the glands were placed directly in the digestion mixture and the iodine was determined as above. The amount of inorganic iodine was found by extracting the powdered glands with distilled water at room temperature for 12–16 hours. It was found there was no increase in the amount of iodine extracted after 8 hours. The filtered extract was then added to the digestion mixture and the iodine determined.

It was necessary to resort to an indirect method for the determination of di-iodotyrosine. The direct methods either involve considerable loss or are impractical for such small amounts as are present in the fetal glands. Harington and Randall ('29) have shown that the total iodine of the adult thyroid gland is in the following combinations: (1) thyroxine and di-iodotyrosine that are bound to protein and are insoluble in water; (2) inorganic iodine, which is water-soluble. Therefore, if the amounts of total iodine, thyroxine iodine, and inorganic iodine are known, the amount of di-iodotyrosine can

be directly calculated. Using this method in two adult glands it was found that the di-iodotyrosine iodine amounted to 60 and 68% of the total iodine. This compares favorably with the results of Brand and Kassel ('39) who used a photometric method involving hydrolysis with alkaline stannite and found that the di-iodotyrosine iodine amounted to 61% of the total iodine in the adult gland.

All of the reagents were purified as outlined by Trevorrow and Fashena ('35). The sodium thiosulfate was kept in an ice-box and standardized daily against a solution of potassium iodate. The iodate standard was checked regularly against a freshly-prepared solution. A known determination was run with every five unknowns. In determining the iodine in the earlier fetal glands, many of which were iodine-free, a blank analysis was substituted for the known solution. Using these precautions recoveries of 100, 98, 105, 100% were obtained on known potassium iodate solutions; recoveries of 103, 101, 100% on known crystalline thyroxine solutions; a recovery of 95.4% on a known amount of thyroxine plus embryo muscle.

Since more than 2000 fetal glands were used in these determinations, the results represent averages rather than individual values. Several glands of uniform size from different litters were used in each analysis. This is particularly true of the earlier fetuses. In one instance (glands from fetuses having a crown-rump length less than 6 cm.) more than 100 glands were used in a single determination. This was necessary in order to obtain about 6 micrograms of iodine in the final determination, since the method is less accurate for quantities far removed from this value.

RESULTS

Chemical. The values obtained for total iodine, thyroxine iodine, inorganic iodine and di-iodotyrosine iodine are presented in table 1 and chart I. The thyroxine iodine values were obtained from a series of glands collected in the spring. The inorganic iodine values, however, were obtained from a series of glands collected in the fall. The marked seasonal

TABLE 1

Total iodine content and the percentages of thyroxine iodine, inorganic iodine, and di-iodotyrosine iodine in fetal and adult thyroid glands.
The numbers in parenthesis in the column marked "Range" indicate the number of analyses in each group

C.R. LENGTH	AGE IN DAYS	AV. DRY WEIGHT PER GLAND	TOTAL IODINE (MICROGRAMS PER WHOLE GLAND)				INORGANIC IODINE PER CENT OF TOTAL IODINE				THYROXINE IODINE PER CENT OF TOTAL IODINE				DI-IODOTYROSINE IODINE PER CENT OF TOTAL IODINE
			Spring		Fall		Range		Range		Range		Range		
			Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range			
(cm.)		(gm.)													
6	42	0.00025	0	—	(1)	—	—	—	—	—	—	—	—	—	—
6-8	42-50	0.00055	0.025	0.014-0.037	(3)	0.021	—	(1)	100	—	(1)	—	0	0 (5)	0
8-9	52	0.001	0.090	—	(1)	0.059	0.049-0.069	(2)	57	—	(1)	—	21	12-31 (4)	22
9-10	53	0.0015	0.170	0.150-0.225	(3)	0.40	0.36-0.44	(2)	22	16-35	(4)	—	63	61-64 (2)	15
10-14	54-66	0.003	1.5	1.0-1.7	(9)	3.0	2.7-3.3	(2)	25	23-27	(2)	—	33	25-48 (6)	42
15-19	68-90	0.013	11	10.8-11.1	(2)	27	26.9-27.5	(2)	9	8.4-10.6	(2)	—	23	18-29 (5)	68
20	92	—	—	—	—	22	15-28	(4)	5	3.3-8.3	(4)	—	—	—	68
20-25	92-100	—	—	—	—	40	35-44	(2)	63	—	(1)	—	—	—	10
26	102	0.019	22.5	21.24	(2)	—	—	—	—	—	—	—	27	18-59 (6)	—
25-30	100-114	—	—	—	—	70	65-73	(3)	47	29-59	(8)	—	—	—	26
	(birth)	—	—	—	—	60	58-62	(3)	85	77-93	(2)	—	—	—	0
Adult I			3100	3000-3200	(2)								30	29-30 (2)	
Adult II			2400	2300-2500	(2)								31	29-33 (2)	
Adult III						8400	8300-8500	(3)	15	14-16	(9)	—	26	— (1)	59
Adult IV						6300	6100-6500	(4)	6	5.9-6.4	(7)	—	25	— (1)	69

difference in the total iodine content of the glands, which will be discussed later, makes it necessary to tabulate the results as percentages rather than absolute values. Since the total iodine in each series is taken as 100%, the percentage that each iodine compound makes of the total iodine can be compared in the two series.

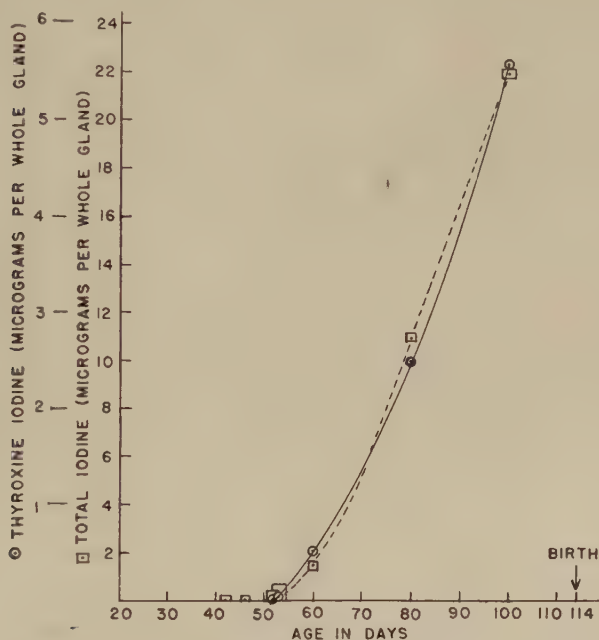


Chart I. The curves show the average thyroxine iodine content of fetal pig thyroid glands measured in micrograms per whole gland (solid line), and the average total iodine content in micrograms per whole gland (broken line).

The gestation period of the pig is 114 days (Warwick, '28). No iodine, organic or inorganic, could be detected in the thyroid glands of fetuses with a crown-rump length of less than 6 cm. (gestation age, 42 days). Iodine was first definitely observed in fetuses with a crown-rump length of 7-8 cm. (46-50 days), and it was found that all the iodine is in the water-soluble or inorganic form when it first appears. Thyroxine iodine and di-iodotyrosine iodine appear simultane-

ously, and in roughly the same amounts, in the thyroids of fetuses with a crown-rump length of 8-9 cm. (52 days). From that age the percentage of total iodine in the form of di-iodotyrosine iodine increases irregularly until near the end of fetal life, when the percentage decreases markedly just before birth. The curves for the amount of total and thyroxine iodine in the glands increase much more regularly.

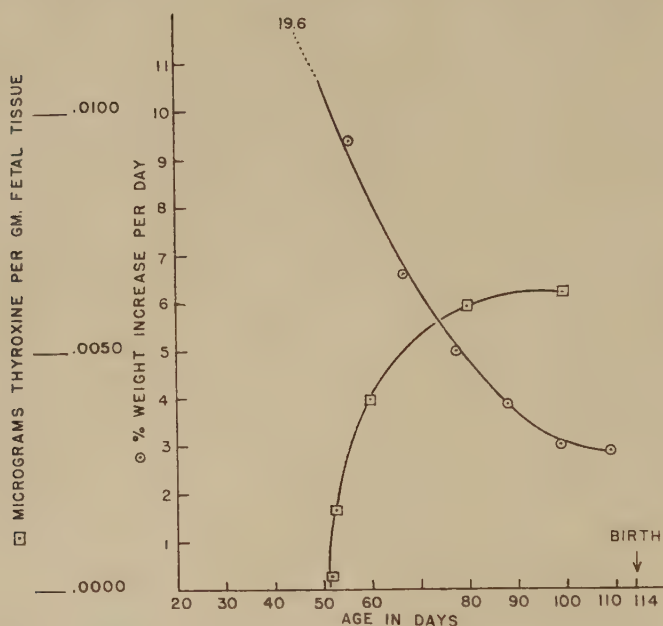


Chart II. The curves show the percentage weight increase per day (solid line) and the average number of micrograms of thyroxine iodine present in the fetal thyroid per gram of total fetal tissue (broken line). It is apparent that there is a general inverse relationship between the curves.

Chart I shows that both the total and thyroxine iodine values increase slowly until a gestation age of about 55 days, after which they rise abruptly. The two curves are nearly coincidental throughout fetal life. Chart II also shows the relative growth curve of the fetal pig, measured as percentage weight increase per day, and the amount of thyroxine iodine in the fetal thyroid, measured as micrograms of thyroxine iodine per

gram of fetus. The data from which these curves were calculated was collected at Beltsville, Maryland, and will be published by Gellhorn, Flexner and Pohl. It appears that the fetal growth is inversely related to the amount of thyroxine in the fetal thyroid. However, since the amount of thyroxine in the fetal thyroid may not be a true indication of the amount of thyroxine available to the fetal tissue, it cannot be concluded from this data that the growth of the fetus is dependent on the function of its own thyroid.

Histological. On gross examination, the thyroid gland of a 5.5 cm. fetus (40 days) appears gelatinous and avascular, almost indistinguishable from the surrounding tissue. The gland slowly becomes more vascular, until in a fetus of 8 cm. (52 days) it suddenly appears as an extremely vascular organ, standing out from the colorless background as a reddish mass.

All the histological preparations used in these observations were fixed in formol-Zenker and stained with hematoxylin and eosin. Microscopically the thyroid of a 5.5 cm. fetus (40 days) consists of a solid unorganized mass of cells (fig. 1, pl. 1). The large vesicular nuclei stand out clearly. The nuclear matrix is clear and pale and contains an extremely coarse chromatin network. Most of the chromatin material in each nucleus is accumulated into five or ten irregular masses. The nucleus occupies roughly a little more than half the volume of the cell, and this relationship persists throughout fetal life. No colloid, acini, or cell cords can be seen. There are small blood vessels filled with red blood cells among the epithelial cells and occasionally red cells lie in the interstices between the gland cells. These cells may be mistaken for colloid, unless they are compared with the cells in the blood vessels.

At the time when thyroxine can first be demonstrated in the thyroid gland (8-9 cm. fetuses; 52 days), the only remarkable histological feature is the striking increase in the blood supply. The cells are indistinguishable from those of the thyroid of a 5.5 cm. fetus (fig. 2, pl. 1). The blood vessels tend to surround chains or clusters of cells and suggest, though indefinitely, the formation of cell cords. No acini or colloid can be seen.

In the thyroid of a 12.0 fetus (60 days) the histological picture is markedly changed (fig. 4, pl. 1). The gland is made up largely of small but definitive and well-formed acini that are filled with pale-staining colloid. The interfollicular cells are morphologically like the cells found in the earlier fetuses. The cells making up the acini, however, are smaller and the nuclei are darker and contain a fine granular chromatin network in addition to the clumps of chromatin material, which are now less prominent. The cytoplasm is still agranular and clear and the cell boundaries are indistinct. No change in the staining properties of the cytoplasm to eosin could be observed. The acini apparently develop by the accumulation of a small amount of colloid among the cells. This colloid droplet is distinctly acidophilic and as it becomes larger the epithelial cells form a single layer around it. By the time the droplet is the size of a single epithelial cell the definitive follicle is apparent. As the follicle grows in size, the colloid stains less deeply in the center of the acinus and the colloid adjoining the cells is more acidophilic, the whole appearing as a pink ring or rim with a pale center. Careful search failed to reveal any empty acini.

In the thyroid of a 26 cm. fetus (102 days) the acini have become larger and more numerous, the colloid more acidophilic and increased in amount. The cells lining the acini are more flattened than those of the previous glands, and the whole structure resembles more closely the adult gland. There is seen in the acini of the glands of fetuses of this age a large number of pale, acidophilic vacuoles. These do not have the clear empty appearance of the vacuoles seen in exophthalmic goitre and unlike them they are scattered throughout the colloid and not clustered around the edge of the colloid near the cells.

DISCUSSION

A correlation of chemical and histological findings shows that the onset of function of the thyroid is immediately preceded by a striking increase in the vascularity of the gland. It is interesting that the blood vessels are present in abund-

ance before the gland begins to function, and are ready to carry away the hormone as soon as secretion begins. It was observed that no cytological changes could be seen with the methods used at the time of the onset of function. It was noted, however, that for a few days after the gland begins to function only a minute quantity of secretion could be detected within it by chemical determination. As soon as the amount of hormone within the gland increases markedly (12.0 cm.; 60 days), striking changes occur. At this time the acini and colloid first appear. As secretory activity increases the gland assumes more and more the adult histological structure.

It has been noted that the percentage of di-iodotyrosine decreases markedly toward the end of fetal life. The colloid at this time contains a large number of pale vacuoles. These structures were not present in the other fetal and adult glands that were fixed and stained routinely in the same manner. It may be that these vacuoles contain either inorganic iodine, which is unusually abundant in the gland at this time, or di-iodotyrosine which is not bound to protein and therefore is water-soluble. In the procedure used it would be impossible to detect water-soluble di-iodotyrosine and it would appear as inorganic iodine.

Various investigators have used different methods to determine the onset of thyroid function in the fetus. Rumph and Smith ('26) reported that the thyroids of fetal pigs having a crown-rump length of 7 cm. did not cause metamorphosis in hypophysectomized tadpoles, but that glands from fetuses with a crown-rump length of 9 cm. (or greater) induced within 2 weeks the changes characteristic of adult thyroids. These observations are in good accord with the results presented in this paper. These investigators also noted that follicles, most of which contained colloid, were present in considerable numbers in the glands of fetuses having a crown-rump length of 9 cm. This is somewhat earlier than the follicles or colloid appeared in the glands examined in this study. Hopkins ('35) reported that the thyroid of 10 day old chick embryos (incubation period, 20 days) causes accelerated metamorphosis in

tadpoles. It has been shown, however, that this test is not specific for thyroxine, since accelerated metamorphosis can be brought about by inorganic iodine if present in sufficient quantities (Lein, '37). Sun ('32) demonstrated iodine in chick embryos of 10 days. Lelkes ('33), analyzing for iodine, showed that it first appears in demonstrable quantities in the thyroid of the human fetus in the fourth month. These investigations in the chick and man and the results presented in this and other papers for the pig suggest that the fetal thyroid begins to function after the end of the first third and before the end of the first half of the gestation period. Kull ('26) could find no colloid or acini or other histological evidence of secretion in the albino rat until just before birth. Observations presented here, however, show that the thyroid may contain thyroxine and di-iodotyrosine in the absence of colloid or acini or other microscopic evidence of secretion.

A comparison of the total iodine in the glands collected in the spring and in the fall (table 1) shows that the total iodine is much higher in the glands collected in the fall than in those collected in the spring. This seasonal variation has been observed by other workers. Fenger et al. ('31) reported that the total iodine content of adult hog thyroids varied from 0.44% in the spring to 0.78% in the fall (calculated on the dry weight). The results presented in the table show that the amount of total iodine is also higher in the fetal glands collected in the fall than it is in the glands of fetuses of the same age collected in the spring. Since the thyroxine iodine series was determined on glands collected in the spring, and the inorganic iodine series was determined on glands collected in the fall, the actual values in micrograms cannot be compared. However, the percentage that the various compounds make up of the total iodine in the gland is relatively constant and can, therefore, be compared. The percentage of total iodine present as thyroxine was found to be only 5% lower in the fall than in the spring. It seems justifiable, then, to use the percentage of thyroxine iodine and the percentage of inorganic iodine in calculating the percentage of di-iodotyrosine iodine.

SUMMARY

1. The thyroid glands of a series of fetal pigs were analyzed for total iodine, thyroxine iodine, and inorganic iodine. The di-iodotyrosine iodine content was calculated by difference from the other iodine values.

2. Iodine was first detected in the thyroids of fetal pigs having a crown-rump length of 7-8 cm., corresponding to a fetal age of 46-50 days. At this time all the iodine was found to be in the water-soluble (inorganic) form.

3. Thyroxine and di-iodotyrosine were first detected in the thyroids of fetal pigs having a crown-rump length of 8-9 cm., corresponding to a fetal age of 52 days.

4. The only marked histological change that was noticed at the beginning of function was the great increase in the vascularity of the gland. At this time only a minute amount of organic iodine could be detected chemically. As soon as the amount of organic iodine compounds became prominent, acini and colloid appeared and the definitive structure of the thyroid became apparent.

It is a pleasure to acknowledge my indebtedness to the Wm. Schluderberg-T. J. Kurdle Company for their cooperation in supplying slaughter house material.

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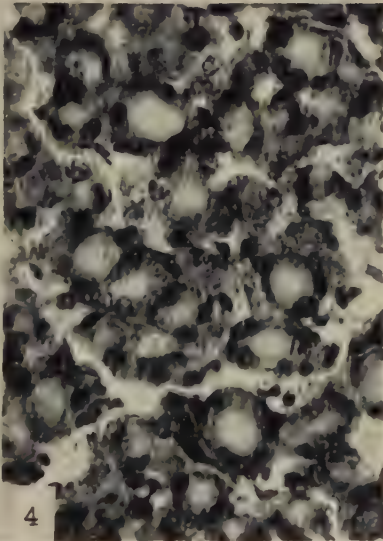
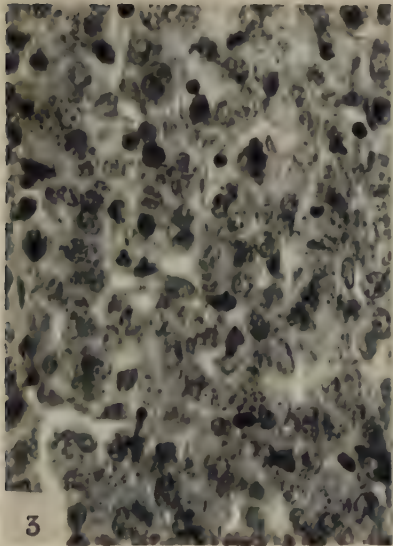
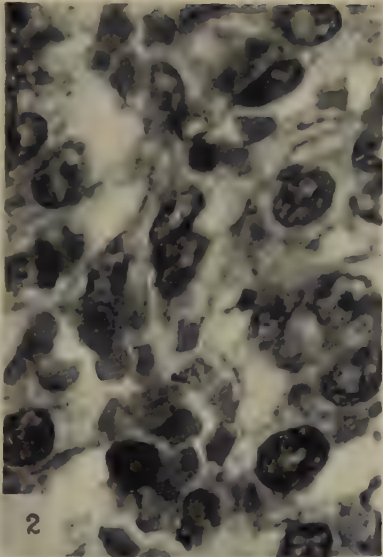
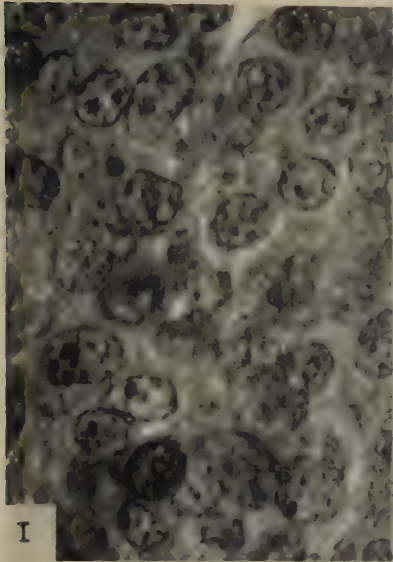
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PLATE 1

EXPLANATION OF FIGURES

- 1 Section through the thyroid of a 5.5 cm. pig fetus (gestation age, 40 days). The gland consists of a solid, unorganized mass of cells. The cells are large and have indistinct cell boundaries. The nuclei are vesicular and contain coarse clumps of chromatin. $\times 1200$. Formol-Zenker; hematoxylin and eosin.
- 2 Section through the thyroid of an 8.5 cm. pig fetus (52 days). The cells are indistinguishable from those of a 5.5 cm. fetus. There is, however, a marked increase in the vascularity of the gland. $\times 1200$. Bouin's; hematoxylin and eosin.
- 3 Section through the thyroid of an 8.5 cm. pig fetus (52 days). The gland is still an unorganized mass of cells. No acini, colloid, or cell cords are present. $\times 530$. Formol-Zenker; hematoxylin and eosin.
- 4 Section through the thyroid of a 12.0 cm. pig fetus (60 days). The striking feature at this time is the presence of numerous, small, definitive follicles. The cells making up the follicles are smaller and stain more deeply than those of the 8.5 cm. fetus. $\times 530$. Formol-Zenker; hematoxylin and eosin.



BOOK REVIEW

PHYSIOLOGY OF THE FETUS, by William Frederick Windle. 1940. Size $6\frac{1}{4} \times 9\frac{1}{4}$ inches. Pages xiii + 249. 27 tables and 70 figures. Index. Cloth. Price, \$4.50. W. B. Saunders and Company, Philadelphia and London.

Dr. Windle's book dealing with the physiological problems which present themselves in the study of prenatal development will prove a most welcome addition to the working library of both physiologists and embryologists. During recent years' investigators in all branches of Anatomy have been pushing out beyond their old limits of descriptive morphology with increasingly critical efforts toward functional correlation and interpretation. The rapid progress occurring concurrently in biological chemistry and physiology has been a great stimulus to such work. In this trend mammalian embryology has been somewhat of a laggard, partly because much of the morphological foundation so necessary to an intelligent undertaking of functional studies was still wanting, and partly on account of the many technical difficulties inherent in making properly controlled physiological studies on organisms with an intra-uterine habitat. Dr. Windle's book comes at an opportune time for the embryologist in that his accumulating knowledge of the structure of growing embryos is rapidly becoming adequate as a secure foundation for superimposed studies utilizing the newer methods of chemistry and physiology. To the physiologist the book should prove stimulating from a different angle. Much of the earlier work of physiologists who have strayed casually into prenatal fields has been less valuable than it might have been had they taken more careful account of the rapidly changing morphology exhibited by all animals in their intra-uterine development. One is tempted to paraphrase to the effect that too often "to them an embryo was an embryo and nothing more." The subtle age changes so characteristic of embryological material have been loosely dealt with, or even as blandly ignored as if embryology were still being interpreted according to medieval doctrines of preformation. Windle's careful assessment of age changes and species differences in interpreting the significance of experimental data should be as constructive for the physiologist as his discussions of newer physiological and chemical methods are for the morphologically minded embryologist.

In the introductory chapter essential fetal-maternal relations are outlined and there is a discussion of the special problems involved in the approaches necessary in working with fetal material. A highly critical attitude in evaluating evidence is established at the outset by the careful analysis here made of the extent to which normal environmental conditions of the fetus are approached by different types of operative procedures.

Chapters II, III and IV, on the fetal cardiovascular mechanism, deal with a field that is especially difficult to handle judiciously at this particular time. The subject bristles with long smouldering controversies of the type which could readily be fanned into exchanges of profitless polemics, as opinion swings one way or another with the acquisition of new, — and often seemingly contradictory, — evidence. Windle has maintained a restrained and open-minded attitude in presenting both the older and the newer phases of the work in this field which is particularly admirable in view of his own active participation in the studies involved. Especially constructive is his recognition of the fact that fluctuating pressures in the umbilical circulation, due to the effects of uterine contraction or relaxation, may quite possibly alter periodically the manner in which blood passes through the fetal cardiovascular system. It would seem that more emphasis might have been placed on this factor in attempting to interpret the significance of the widely varying results obtained by different investigators from determinations of oxygen saturation made at various critical situations in the fetal circulation. Temporary changes in relative pressures may well be involved in a disturbing manner also in injection experiments designed to ascertain the course of blood through the fetal heart, a possibility which Windle suggests but, — perhaps wisely in view of the fragmentary character of our present knowledge as to pressures, — does not attempt to evaluate.

In the field of postnatal circulatory changes the author's first-hand acquaintance with the important work of the Barcroft, Barron, Barclay group of Cambridge, England, makes him a particularly interesting commentator. The probability, as indicated by the recent studies of this group, that functional closure of the ductus arteriosus through muscular action occurs in advance of its leisurely structural closure is most interesting and significant. That functional closure of the foramen ovale occurs months before its anatomical closure has now for some time been widely accepted. A similar situation with regard to the ductus is so appealing on theoretical grounds that a little extra caution in evaluating the evidence is indicated. It should be borne in mind that an initial tendency on the part of the circular smooth muscle of the ductus to contract, does not necessarily

imply a contraction sufficiently strongly and steadily maintained to shut off all blood flow during the 6 to 8 weeks occupied by morphological closure. The dramatic quality of an immediate muscular response should not cause us to forget the importance of the slower but more positive structural closure. In this connection Windle's contention (p. 46) that if the ductus did not close promptly "one should expect to hear the characteristic murmur of a patent ductus in every infant" must be taken with some reservations. Gross in his brilliant paper on ligation of the ductus arteriosus, which appeared subsequently to Windle's writing of this section,¹ has pointed out that even children in whom the ductus had remained so fully patent as to justify surgical intervention might have failed to exhibit any murmur during early infancy. This suggests that a characteristic murmur, even when a patent ductus undeniably exists, may not be audible until a considerable excess of systemic over pulmonary pressure has been built up. It is quite possible that this may not occur until after the time when the ductus would, in the normal course of events, have been anatomically sealed. Certainly the preponderance of the left over the right ventricular wall is developed very slowly, its full degree not being reached until the fourth or fifth year after birth; and the development of this preponderance must be correlated with the increase of systemic above pulmonary pressure. These comments should not be taken as minimizing the possible importance of a sphincter-like action of the muscle of the ductus arteriosus in accelerating postnatal circulatory changes. They merely suggest the necessity of revising our ideas on this subject with the deliberation indicated by the complexity of the interacting processes involved and the still fragmentary character of the information available as to many of their aspects.

Closely related to the matter of a smooth and efficient transition from intra- to extra-uterine conditions in the cardiovascular mechanism is the development of the fetal respiratory system to a degree which prepares it for full functional activity at the moment of birth. Chapter V contains an interesting review of the modern physiological and chemical methods which are just beginning to be brought to bear on the study of the gaseous interchanges between fetus and mother. The intensely interesting matter of respiratory movements on the part of the fetus in utero is considered in Chapter VI. The experimental evidence that is forcing the abandonment of the old idea of complete inactivity of the respiratory mechanism during prenatal life is covered in considerable detail. There is also a good

¹ Gross, R. E. 1940 Experiences with surgical treatment in ten cases of patent ductus arteriosus. *J. A. M. A.*, vol. 115, pp. 1257-1262.

presentation of the present status of the controversy as to whether tentative respiratory movements are regularly carried out, or whether fetal anoxemia precipitates them at irregular intervals. The most interesting thing to most of us, however, will be not the controversial details but the arresting new conception that there may be some sort of tentative "trying out" of the mechanism of respiration even though the fetus is developing submerged in the amniotic fluid. As is the case with the circulatory system, we are beginning to see a respiratory mechanism more fully prepared and tested for its postnatal role than we had previously realized. It is still true that as individuals we crowd into a few crucial moments the change from water living to air living that in phylogeny must have been spread over eons of transitional amphibious existence. But as we learn more about this change in manner of living, it becomes apparent that we should marvel more at the completeness and the perfection of the preparations for its smooth accomplishment, and dwell less on the old theme of the revolutionary character of the changes involved.

In Chapter VII on the fetal digestive system there are assembled observations from many sources which taken together tell the interesting story of intra-uterine swallowing reactions as they involve the amniotic fluid. Here Windle seems momentarily to relax his usual highly critical attitude when he presents De Snoo's somewhat startling method of treating polyhydramnios, without at least commenting that the facts rested on very few cases, and the conclusions drawn from them seem a bit bold. When one reflects that the fetus is apparently accustomed to imbibing amniotic fluid composed in part of urine, with a dash of vernix caseosa, and possibly at times some meconial thickening, one should not be accused of being obnoxiously skeptical for wondering just a little as to the effect of adding a touch of saccharine to such a mixture. Would a little sweetening of the amniotic fluid really make it so much more palatable that a fastidious fetus which had hitherto not been drinking freely would promptly respond by drinking so much more that the hydramnios would be effectively reduced? The facts cited in evidence, namely that saccharine was recovered from the blood and urine of the fetuses, prove no more than that amniotic fluid with whatever it happened to contain had been swallowed. This no one would doubt. What one would like to know more about is the alleged response to the saccharine.

Chapter VIII on the kidneys, fetal fluids, and skin brings together in interesting fashion an extremely varied mass of material that with less skillful handling could easily have been a mere jumble of ideas. Particularly interesting is the suggestive opening of the almost

untouched field of the changes in the mechanism of elimination at the time of birth.

To one lacking first-hand experience with the meticulously detailed observations necessary to make any sound progress in functionally correlated studies on the developing nervous and muscular systems, there is likely to be some feeling of disappointment in reading Chapters IX–XIII which deal with this field. Much that one would like to know more explicitly is merely suggested in sketchy outline or left untouched. That, however, is the state of our present knowledge of the field. It is to Windle's credit that he scrupulously refrains from the temptation to fill in even the most intriguing of these gaps by conjecture. In passing one cannot refrain from commenting that this temptation to paint in blank spaces impressionistically always seems to be peculiarly difficult to resist when the nervous system is concerned. These chapters should be provocative in their clean-cut presentation of the start that has been made in this difficult field and even more so in their outlining of the tremendous gaps that need to be filled by further investigation.

The two final chapters deal with the endocrine glands, and with fetal nutrition and metabolism. The reader cannot fail to be appreciative of the enormous amount of material collected from widely scattered sources and here organized into logical sequence and critically analyzed.

Thinking back over the book as a whole, one realizes that its simple unpretentious style is well adapted to its objective. Extraordinarily few typographical errors are encountered and the figures for the most part have been well selected, although a few of them have been somewhat too much reduced so that consulting them requires an unnecessary struggle to see details. A carefully made index adds greatly to the general useability of the book. Perhaps above all one is impressed by the tremendous range of subjects covered and the careful documentation of the statements made,—particularly in controversial fields. The resulting lists of references at the ends of the chapters will be of particular interest to investigators. This reviewer, however, keenly regrets the employment of that form of citation which omits the title of the article. It makes a bibliography much less satisfactory to consult at the time of reading, and greatly lessens its value as a readily utilizable source of new material for one's own working files. The only justification for using such an abbreviated method of citation would seem to be the shortening of references to the point where they could appear as footnotes on the page one is reading. If one is forced to turn to a special bibliographical section, one is certainly entitled to find the references complete with titles. One resents being forced to go to the

library for information that the author readily could have placed at one's immediate disposal if the publisher had not been niggardly.

There is another matter in connection with the manner of giving citations that seems to call for comment. In the case of collaborative papers, the fact that one who takes the trouble to follow the text reference back to the bibliography can usually find fuller citation, does not seem sufficient justification for giving text or figure references by mentioning only the name of the senior author and relegating his collaborators to the anonymity of "et al." Not infrequently the major part of the actual work has been done by a junior author and the more junior he is the more important to him is proper recognition. Similarly, the fact that someone's original drawing appealed sufficiently to the writer of a textbook so that it was incorporated in one of his plates should not relieve one who subsequently borrows from the first borrower from crediting the original source.

These minor criticisms should receive only passing consideration. What is of importance is that Dr. Windle has made available to us a book which ably covers an extraordinarily wide field in brief, readable, and richly documented form. Moreover, much of the field lies in what has been a sort of no-man's-land between two conventional disciplines. The wealth of the provocative new material assembled in it should prove a real stimulus to students and investigators in both physiology and embryology.

BRADLEY M. PATTEN

REVIEWS OF APPROVED FILMS IN ANATOMY AND BIOLOGY

The following films in biology have been approved by one or more members of the Committees of the American Association of Anatomists or the American Society of Zoologists, and The Wistar Institute Committee.

THE EARLY DEVELOPMENT OF THE RABBIT EGG IN VITRO

WARREN H. LEWIS AND P. W. GREGORY

(400 feet, monochrome, silent)

This film gives an exquisite picture of the early development of the rabbit ovum in vitro. It shows with extraordinary clearness the early division of the ovum from one to many cells and the formation of the trophoblast and segmentation cavity. The ova were obtained by washing out the tubes of mated rabbits, and were kept alive in auto- or homo-plasma on a glass slide. Pictures were taken at 24-second intervals.

This film achieves much more than the providing of a picture of the early embryonic stages, for it brings out many points which would probably never have been observed without the motion picture records, such as the agitation of the cytoplasm before division, the changes in shape or "pulsations" of the cells, and the periodic expansions and contractions of the ovum after the formation of the segmentation cavity. Since many of the scenes are timed by means of a clock, the film forms valuable research material.

Altogether, both for research and teaching purposes, it would be difficult to praise this film too highly. ★ A — E. R. CLARK.

A description has been published in *Science*, 1929, vol. 69, no. 1782, pp. 226-229.

SHORT FILM ON THE EARLY DEVELOPMENT OF THE RABBIT EGG

WARREN H. LEWIS AND P. W. GREGORY

(80 feet, monochrome, silent)

In order to make available the story of the early development of the rabbit egg to those unable to acquire the full reel, Lewis and Gregory have arranged this shorter (3 minute) film using scenes taken from the longer reel (q. v.). ★ A — E. R. CLARK.

TUMOR CELLS AND MACROPHAGES IN TISSUE CULTURES. RAT SARCOMAS AND CARCINOMAS

WARREN H. LEWIS

(360 feet, monochrome, silent)

This film presents an extraordinary picture of the behavior, in tissue cultures, of cells derived from a variety of rat sarcomas and carcinomas, all taken with time-lapse apparatus. Binucleate and trinucleate cells are shown; also several remarkably clear pictures of mitotic division. In all scenes there are macrophages and lymphocytes in action, while one scene depicts the phagocytosis of dead cells by macrophages.

This is an extremely valuable film, both for research and teaching. The photography is superb, legends satisfactory, length of scenes well-chosen. ★ A — E. R. CLARK.

MESENTERIC LYMPHATICS, THEIR CONDUCT AND THE BEHAVIOR OF THEIR VALVES IN THE LIVING RAT

RICHARD L. WEBB

(400 feet, monochrome, silent)

It would be difficult to overestimate the value and importance of this film. It shows in most striking fashion the contractility of the mesenteric lymphatics in the rat. That the mesenteric lymphatics in rats and guinea pigs aid in the movement of lymph by contracting has been described in the literature (Heller, 1869; Lieben, '10; Florey, '27). Thus the demonstration of their contractions by means of motion pictures does not represent a new discovery. However, the written descriptions have attracted but little notice, while Doctor Webb's motion pictures are so vivid and striking and present so many interesting details of behavior of valves, and of the nature of lymph movement, in addition to the tempo and characteristics of the contractions, that they carry the story in such a way as to leave an unforgettable impression.

The film is of interest both for research and teaching. It will be of exceptional interest to physiologists, anatomists, pathologists and clinicians.

To avoid possible misconceptions, it would be well, if used as a teaching film, to add a strip stating that this contractility has been found only in rat and guinea pig; that eight other mammalian species investigated by Florey showed no contractions of mesenteric lymphatics; and that nothing is known regarding the contractility of these lymphatics in the human. ★ E — E. R. CLARK.

Motion pictures showing contractions of mesenteric lymphatics were shown by Webb at the 1934 session of the American Association of Anatomists. *Anatomical Record*, vol. 58, 1934, supplement, p. 95.

Other references: Heller, A., 1869, *Zbl. Med. Wiss.*, vol. 7, p. 545; Lieben, S., '10, *Zbl. Physiol.*, vol. 24, p. 1164; Florey, H., '27, *J. Physiol.*, vol. 62.

THE EFFECT OF VARIOUS DRUGS ON CONTRACTIONS OF MESENTERIC LYMPHATICS IN THE RAT

RICHARD L. WEBB

(300 feet, monochrome, silent)

This is an important film on a subject of great interest, which has been little investigated. It is worthwhile both as a research and as a teaching film. The photography is good, length of scenes well-chosen, and explanatory titling excellent.

The various scenes show the effect, on the contractible mesenteric lymphatic vessels of the albino rat, of the following substances: nembutal, pitressin, pitocin, adrenalin, pilocarpin, barium chloride and nitroglycerine.

If used for teaching it would be well if it could be preceded by Doctor Webb's film showing contractions of mesenteric lymphatics in the rat.

This film will interest pharmacologists, physiologists, anatomists and clinicians. ★ E — E. R. CLARK.

The film was demonstrated at the 1935 meeting of the American Association of Anatomists, see *Anatomical Record*, 1935, vol. 61, supplement, p. 64.

★ Copy deposited in the Film Depository of The Wistar Institute, and is available to all scientists for use at The Wistar Institute.

A — Available for sale or rent by The Wistar Institute.

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SUPPLEMENT

WAYNE JASON ATWELL
1889-1941

IN MEMORIAM ¹

In the minds of all anatomists the name of Wayne Jason Atwell is associated with the hypophysis. His study of this complex organ, to the knowledge of which he made many important contributions, began when he was a graduate student with Huber at the University of Michigan, a quarter of a century ago, and continued until terminated by death. So recent and unexpected was this sad event that many of you no doubt came to this meeting expecting to hear Atwell speak, in his usual calm and convincing manner, about the hypophysis, as listed in the program for this morning. The abstract for this paper shows beautifully the thoroughness with which he worked and why his words carried conviction.

Atwell's investigations were not limited to the hypophysis; other aspects of endocrinology also received attention, and his outstanding publication in other fields is the masterly study and excellent description of a 17-somite human embryo. All of his investigations were made by the method most of us have to use—painstaking careful work month after month and year after year. Atwell knew how to evaluate the evidence thus found, and his contributions will endure.

Wayne Atwell was born October 19th, 1889, at Fairfield, Nebraska, attended public schools in that prairie State and obtained his baccalaureate degree at Nebraska Wesleyan in 1911. After a year in medical school at Michigan, limited financial resources caused Atwell to seek an assistantship.

¹ Presented at the fifty-seventh annual meeting of the American Association of Anatomists, at Chicago, April 9, 1941.

During the 5 years that he was on the staff of G. Carl Huber's department, he developed a permanent interest in anatomy, and demonstrated his ability by earning the degrees of A.M. and of Ph.D., in spite of a very heavy teaching load.

Before his twenty-ninth birthday, in the autumn of 1918, Dr. Atwell became Professor of Anatomy at the University of Buffalo, and had to face problems that would have baffled many older and more experienced anatomists. Emphatically it can be said that Atwell made good in all aspects of the difficult task. Ever since he took charge there has been a steady stream of substantial papers bearing the footnote "From the Department of Anatomy, The University of Buffalo"; and the majority of the papers have been by other staff members, for whom Atwell provided the opportunity to carry out their investigations. Atwell was also a good teacher. He knew how to impart knowledge and, by his own example, showed his students how good work is done. His colleagues in other departments, as well as his students, liked and respected him, and both groups soon found he could be relied upon in all efforts to improve conditions for their work. In order to have a better understanding of the clinical problems connected with his own investigations and to have a better grasp of the broad problems of medical education, Atwell took courses in the other departments and received the M.D. degree at Buffalo in 1934. From being the youngest professor, Dr. Atwell grew into one of the most influential and important members of the faculty of his school.

Atwell's second publication, on a simple method to convert a photograph into a line drawing, well illustrates two characteristics that impressed all who were closely associated with him; namely, his ingenuity with apparatus and technical procedures, and his ability to adapt himself to the necessities of a situation. He enjoyed working with his capable hands as well as with his brain, and was never happier than when building something, whether a piece of laboratory equipment, a wax model, furniture, or even a house. He liked outdoor life, preferred camping trips for vacations, and was proud of his

garden, which he took care of himself. Unlike many scientists of the present day, Dr. Atwell maintained his boyhood interest in religion and participated especially in the activities of the children and young people of his church.

Atwell was elected to membership in the American Association of Anatomists at the St. Louis meeting in December, 1914, and attended the majority of the meetings that have been held since then. He was one of our most reliable and dependable members, and at the time of his death was serving us as a member of the Executive Committee and as an Associate Editor of *The American Journal of Anatomy*. His death on March 27th of this year, at the early age of 51 years, was a serious loss not only for the University of Buffalo but also for the American Association of Anatomists, and we join with his bereaved family in grief and mourning.

STACY R. GUILD

THE JOHNS HOPKINS UNIVERSITY

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

FIFTY-SEVENTH SESSION

*The University of Chicago,
Chicago, Illinois
April 9, 10 and 11, 1941.*

The Fifty-seventh Meeting of the Association, by invitation of The University of Chicago, as a part of the Celebration of the Fiftieth Anniversary of the University, was held in the buildings of The University of Chicago, Wednesday April 9, Thursday April 10 and Friday April 11, 1941. The Local Committee was comprised of G. W. Bartelmez, William Bloom, W. M. Krogman, F. Wassermann, S. Polyak, M. H. Knisely, Sylvia H. Bensley, D. Clark, I. Rossman, Paul C. Weiss and Charles H. Swift, Chairman.

Attendance

	Total registered 484		
Association members	350 (?)	Out of town	380
Non-members	134 (?)	Local	104

RESUME OF THE SCIENTIFIC PROGRAM

The Fifty-seventh Session of the American Association of Anatomists was opened in general session by President Philip E. Smith at 10:00 A.M., in Mandel Hall. Following the announcement of Auditing and Nominating Committees, nine scientific papers were presented. A tenth paper, by Wayne J. Atwell, had been scheduled but was withdrawn because of Dr. Atwell's untimely death, which occurred less than 2 weeks before the meeting. After the reading of the papers the Association in general session paid tribute to Dr. Atwell with the reading of a testimonial by Dr. Stacy R. Guild, once a classmate of Dr. Atwell at the University of Michigan.

The remainder of the scientific sessions for the reading of papers was divided into three concurrent sessions, except for Thursday morning and afternoon, when the number was increased to four, and the Friday afternoon general session. The Thursday morning session extended from 9:30 to 12:15, the Friday morning session from 9:30 to 12:30, while the Wednesday and Thursday afternoon sessions were restricted to an hour and a half (2:00 to 3:30). Demonstrations were held from 3:30 to 5:30 on both Wednesday and Thursday afternoons. The motion pictures were treated in two categories, as "papers" and as "demonstrations". Those given as "papers" were presented at the regular sessions for reading of papers. Of those rated as "demonstrations" four were scheduled from 4:10 to 5:30 Wednesday, and four at the same time on Thursday. Two of the motion pictures scheduled for Thursday were not shown, although the authors were present and prepared to present them. Objection to their showing was raised because of the fear that, on account of the nature of the films, serious difficulties might arise unless the audience were rigidly restricted to members of the Association—a provision which has not yet been enforced in the meetings of the Association. On the other hand, an unscheduled film was shown on Wednesday afternoon which had been sent to this country from England and received too late for inclusion in the program. It was entitled: "X-ray cinematograph studies on the closure of the ductus arteriosus", by Barclay, Barcroft, Barron and Franklin. It was presented by D. H. Barron, University of Missouri.

The scheduled number of "papers from platform" was 138, of which 11 were withdrawn, leaving a total of 127 papers presented. This compares with 132 scheduled and 124 presented in 1940. There were 32 papers "read by title" compared with 42 last year. Forty-nine of 50 scheduled demonstrations materialized.

The scientific sessions closed with an invitation program Friday 2:00 to 4:00 P.M., at which the following addresses were given:

Dr. Warren H. Lewis: "Cell division."

Dr. Robert Chambers: "The role of calcium in the permeability of cellular membranes."

Dr. C. C. Macklin: "Some features of the functional anatomy of the lung."

The Presidential address, usually given in the first year of the 2-year term, was postponed to 1942 on account of the illness of President Smith during a part of the Fall of 1940.

SOCIAL ACTIVITIES

Banquet. Following the Dinner, which was attended by 207 persons, President Smith, acting as toast-master, congratulated the University on its Fiftieth Anniversary, and expressed the wish that the second 50 years might be as productive as the first. The Association was welcomed to the University by Professor G. W. Bartelmez. Professor Donald B. Phemister, an invited guest, gave a stimulating talk on the relations between Anatomy and Surgery. Among other suggestions he urged Anatomists to make provision for the teaching of the anatomy of special regions to graduates equipping themselves for the various surgical specialties. Another invited guest, Professor A. J. Carlson, proposed serious consideration of the advisability of bringing human anatomy to the colleges and perhaps even to the high schools, in order to strengthen health education. The festivities closed with a motion picture demonstration by Vice-President C. G. Hartman, which was introduced as "The intimate family life of the wasp".

Smoker. The Smoker on Wednesday evening, at which the members of the Association were guests of The University of Chicago, was attended by approximately 350 persons.

Entertainment. Women in attendance at the meeting were entertained delightfully at a Tea at the home of Mrs. G. W. Bartelmez on Thursday afternoon. Many members took advantage of the opportunity to visit museums and other places of interest which abound in Chicago.

BUSINESS SESSIONS

WEDNESDAY, APRIL 9, 1941

10:00 a.m. At a brief Business Session President Smith announced the appointment of the following Committees:

Auditing Committee: Professor William Bloom, Chairman, Professor Charles M. Goss.

Nominating Committee for 1942: Professor Edward A. Boyden, Chairman, Professor Carl G. Hartman, Professor Joseph C. Hinsey.

THURSDAY, APRIL 10, 1941

12:15 p.m. Principal Business Session, President Philip E. Smith in the chair. The President reported that the minutes of the Fifty-sixth Session were printed in *The Anatomical Record*, vol. 76, no. 4 (Supplement no. 3), April 25, 1940, pp. 1-7 and 10-28. On motion, duly made and seconded, the minutes were approved as printed.

Report of the Treasurer: The Secretary-Treasurer made the following report for the year 1940:

Financial statement for year 1940

Balance on hand in Checking Account, Dec. 31, 1939, last audit of accounts.	\$1,017.98	
Receipts from dues and abstracts	1,119.62	
<i>Total Credits</i>		\$2,137.60
Expenditures, 1940:		
Postage, stationery, supplies and miscel.	136.16	
Printing	51.95	
Programs, 56th Meeting	90.00	
Secretarial assistance	50.00	
Travelling expenses, Secretary	50.04	
Wistar Institute (abstracts)	193.35	
Local Committee (56th Meeting)	150.00	
First National Bank (returned checks)	5.16	
<i>Total Expenditures</i>		726.66

Balance on hand Dec. 31, 1940, and deposited in the name of the American Association of Anatomists in the First National Bank, Philadelphia, Pa.		\$1,410.94
Special Interest Account #40050, First National Bank, Philadelphia, Pa.		
Balance December 31, 1939	677.84	
Interest to June 7, 1940	2.68	
Transferred to Interest Account No. 10244, Morris Plan Bank, Philadelphia, deposited June 17, 1940	680.52	
Interest to December 31, 1940	5.67	
Balance December 31, 1940		686.19
<i>Total Balance</i>		<u>\$2,097.13</u>

It will be noted that the checking account gained \$392.96 and the Special Account \$8.35, making a total gain for the year of \$401.31. The unusually large gain results from the collection of increased dues to cover abstracts which will have been paid for before the 1941 meeting, at a cost of fifty cents per member, a total of \$342.00. This leaves a net gain of \$59.31.

Report of the Auditing Committee: Professor Bloom moved that the sum of \$3.93, occurring on check no. 27, January 11, 1940, which had not been received by the Bank, be added to income and that the check be cancelled. This motion was seconded and passed.

Professor Bloom then read the following report: "The undersigned Auditing Committee has examined the accounts of the Secretary-Treasurer for the year 1940, and finds total receipts reported to be those entered in the Journal, expenditures in accordance with receipted bills, and the balance on hand December 31, 1940 in agreement with the bank statement of that date and pass book of Special Account."

(Signed) WILLIAM BLOOM
CHARLES M. GOSS

On motion, duly seconded, the reports of the Treasurer and of the Auditing Committee were accepted and adopted.

Election of Officers: In the absence of Professor George W. Corner, Chairman of the Nominating Committee, Professor W. H. F. Addison, for the Committee of which Professor A. T. Rasmussen was the third member, placed the following nominations before the Association:

For Members of the Executive Committee, for the term expiring in 1945, as specified in the Constitution: Professor E. Horne Craigie and Professor Walter E. Sullivan.

The President called attention to the provision of the Constitution permitting additional nominations if handed to the Secretary in writing by any five members. As no additional nominations were forthcoming, a motion was made, seconded and carried that the Secretary cast a unanimous ballot for the above-named nominees.

Election of Representatives: The Secretary read, in behalf of the Executive Committee, the following nominations: Representative on the Commission for Standardization of Biological Stains for 1 year: Dr. Harold A. Davenport; Representatives to the Council of the American Association for the Advancement of Science for 1 year: Professor F. H. Swett, Professor J. T. Patterson, and Professor G. W. Bartelmez; Delegate to the Union of Biological Societies for a term of 3 years: Professor Stacy R. Guild—the terms of service being those specified in the Orders of the Association.

In the absence of other nominations, the above nominees were duly elected.

New Members: The Secretary presented the following forty-four names of persons recommended by the Executive Committee for election to membership in the Association, the names in parentheses indicating the sponsors:

- ABERCROMBIE, WARREN F., A.B., Sc.M., Ph.D., Associate Professor of Biology, *Howard College, Birmingham, Ala.* (C. J. Sandstrom, H. W. Stunkard.)
- ADAMSTONE, F. B., B.A., M.A., Ph.D., Associate Professor of Zoology, *University of Illinois, Urbana, Ill.* (W. Shumway, L. A. Adams.)
- ALEXANDER, WILLIAM F., B.S., M.S., Ph.D., Instructor in Anatomy, *Louisiana State University, School of Medicine, New Orleans, La.* (A. Kuntz, B. I. Burns.)
- ALTSCHUL, RUDOLF, M.D., Instructor in Anatomy, *University of Saskatchewan, Saskatoon, Canada.* (D. G. Revell, J. L. Jackson.)
- BEATON, LINDSAY E., A.B., B.M., M.D., M.S., National Research Council Fellow, 1940–41, *Northwestern University Medical School, Chicago, Ill.* (B. J. Anson, S. W. Ranson.)
- BECKER, ROLAND F., B.S., M.S., Ph.D., Instructor in Anatomy, *Northwestern University Medical School, Chicago, Ill.* (W. F. Windle, L. B. Arey.)

- BÉLANGER, LEONARD F., A.B., M.D., A.M., Assistant, *Department of Histology and Embryology, University of Montreal, Montreal, Canada.* (G. B. Wislocki, J. L. Bremer.)
- BLOUNT, ISABEL H., A.B., A.M., Ph.D., Voluntary Research Worker, *Department of Anatomy, University of Minnesota, Minneapolis, Minn.* (A. T. Rasmussen, C. M. Jackson.)
- BOYER, ESTHER L., M.A., Ph.D., M.D., Instructor in Anatomy, *Woman's Medical College of Pennsylvania, Philadelphia, Pa.* (H. Kuhlenbeck, R. N. Miller.)
- BRADBURY, JAMES T., B.S., M.S., Sc.D., Endocrinologist, *Bureau of Dairy Industry, U. S. Department of Agriculture, Beltsville, Md.* (E. T. Gomez, C. W. Turner.)
- CANDELA, POMPEO B., M.D., Research Associate in Anatomy, *New York Medical College, New York City.* (T. H. Evans, C. E. Tharaldsen.)
- CLAUSEN, HARRY J., A.B., M.S., Ph.D., Instructor in Anatomy, *University of Colorado School of Medicine, Denver, Colo.* (I. E. Wallin, A. R. Buchanan.)
- DALTON, ALBERT J., B.S., M.A., Ph.D., Lecturer in Histology, *McGill University, Montreal, Canada.* (C. P. Martin, Hans Selye.)
- EASTLICK, HERBERT L., A.B., M.S., Ph.D., Assistant Professor of Zoology, *Washington State College, Pullman, Wash.* (V. Hamburger, L. J. Wells.)
- EMERSON, HENRY S., B.A., Ph.D., Instructor in Anatomy, *University of Michigan, Ann Arbor, Mich.* (B. M. Patten, E. C. Crosby.)
- EMMEL, VICTOR M., A.B., Sc.M., Ph.D., Instructor in Anatomy, *The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.* (K. E. Mason, Edgar Allen.)
- FOGG, LLOYD C., B.S., Ph.D., Assistant Professor of Histology and Embryology, *Boston University Medical School, Boston, Mass.* (A. S. Woodman, J. L. Conel.)
- GATZ, ARTHUR J., B.A., M.A., Ph.D., Instructor in Zoology, *Carleton College, Northfield, Minn.* (A. R. Ringoen, E. A. Boyden.)
- GRAY, MARY E., B.A., Ph.D., Assistant, *Department of Anatomy, Vanderbilt University, Nashville, Tenn.* (S. L. Clark, E. H. Tompkins.)
- HALL, B. VINCENT, A.B., Ph.D., Assistant Professor of Zoology, *University of Illinois, Urbana, Ill.* (L. A. Adams, W. Shumway.)
- HARE, WILLIAM K., A.B., M.S., Ph.D., Assistant Professor of Anatomy, *Cornell Medical College, New York City.* (J. C. Hinsey, S. W. Ranson.)
- HILL, JOHN E., Ph.D., Assistant Curator of Mammals, *American Museum of Natural History, New York City.* (A. B. Howell, E. R. Hall.)
- LANIER, RAYMOND R., B.A., Ph.D., Assistant in Anatomy, *Washington University, St. Louis, Mo.* (R. J. Terry, M. Trotter.)
- LEATHEM, JAMES H., B.S., M.A., Ph.D., Research Associate in Anatomy, *Columbia University, College of Physicians and Surgeons, New York City.* (P. E. Smith, S. R. Detwiler.)
- LIMSON, MARCIANO, M.D., Associate Professor of Anatomy, *College of Medicine, University of the Philippines, Manila, P. I.* (A. Garcia, F. Cuajunco.)
- MILLER, JAMES A., A.B., Ph.D., Instructor in Anatomy, *University of Michigan, Ann Arbor, Mich.* (B. M. Patten, E. C. Crosby.)
- MILLER, ROLAND E., B.S., M.S., Ph.D., Assistant Professor of Anatomy, *School of Medical Sciences, Wake Forest College, Wake Forest, N. C.* (J. A. Cameron, M. D. Overholser.)

- MINCKLER, JEFF, A.B., M.A., Ph.D., Instructor in Anatomy, *Creighton University, Omaha, Neb.* (C. M. Jackson, A. T. Rasmussen.)
- NELSON, ROSE C., B.A., M.A., Ph.D., Associate Fellow and Instructor in Pediatric Research, *Children's Hospital Research Foundation, Cincinnati, O.* (C. K. Weichert, I. G. Schmidt.)
- OSBORN, CLINTON M., A.B., A.M., Ph.D., Instructor in Histology and Embryology, *Department of Anatomy, Ohio State University, Columbus, O.* (L. F. Edwards, R. A. Knouff.)
- PICK, JOSEPH, M.D., Instructor in Anatomy, *New York University, College of Medicine, New York City.* (D. Sheehan, W. J. Krieg.)
- PLAGGE, JAMES C., S.B., Ph.D., Instructor in Gross Anatomy, *University of Maryland School of Medicine, Baltimore, Md.* (E. Uhlenhuth, C. L. Davis.)
- REECE, RALPH PARLETTE, B.Sc., M.Sc., Assistant Professor of Dairy Husbandry, *New Jersey Agricultural Experiment Station, New Brunswick, N. J.*
- RICHARDSON, DOROTHY, A.B., Ph.D., Associate Professor of Zoology, *Rockford College, Rockford, Ill.* (A. E. Adams, S. R. Detwiler.)
- DE ROBERTIS, EDUARDO D. P., Member of Staff of Institute of General Anatomy, University of Buenos Aires, Argentina; Rockefeller Fellow, *Department of Anatomy, Johns Hopkins Medical School, Baltimore, Md.* (I. Gersh, W. L. Straus, Jr.)
- ROBERTS, JOSEPH T., B.A., M.S., M.D., Instructor in Anatomy, Instructor in Medicine, Assistant in Pediatrics, *University of Texas, Medical Branch, Galveston, Tex.* (D. Duncan, H. Cummins.)
- ROSSMAN, ISADORE, A.B., Ph.D., Research Assistant in Anatomy, *University of Chicago, Chicago, Ill.* (R. R. Bensley, G. W. Bartelmez.)
- TERRILL, CLAIR ELMAN, B.S., Ph.D., Physiologist and Geneticist, *U. S. Western Sheep Breeding Laboratory, Dubois, Idaho.*
- THOMSON, STEWART CRAIG, A.B., M.D., M.S., Assistant Professor of Anatomy, *Loyola University School of Medicine, Chicago, Ill.* (T. T. Job, R. M. Strong.)
- WARREN, CHARLES O., A.B., Ph.D., M.D., Research Associate in Anatomy, *Cornell Medical College, New York City.* (J. C. Hinsey, C. V. Morrill.)
- WHITAKER, WAYNE L., A.B., A.M., Ph.D., Instructor in Anatomy, *University of Michigan, Ann Arbor, Mich.* (B. M. Patten, E. C. Crosby.)
- WRIGHT, WALTER H., D.D.S., B.S., M.S., Ph.D., Professor of Anatomy and Prosthetic Dentistry, *School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* (J. C. Donaldson, I. D. Hogg.)
- ZACHARIAS, LEONA RUTH, B.A., M.A., Ph.D., Instructor in Anatomy, *Columbia University, College of Physicians and Surgeons, New York City.* (S. R. Detwiler, W. M. Copenhaver.)
- ZEIT, WALTER, B.S., M.S., Ph.D., Assistant Professor of Anatomy, *Marquette University School of Medicine, Milwaukee, Wis.* (E. J. Carey, W. E. Sullivan.)

On motion, duly seconded, the Secretary was instructed to cast a ballot for all candidates proposed by the Executive Committee, and they were declared elected.

Members placed in the category of "members relieved of the payment of dues": The Secretary reported that, by vote of the Executive Committee, nineteen members had been placed in this category in accordance with the provision of Article VI, Section II of the Constitution, adopted at the Fifty-sixth Session of the Association. It was pointed out that this regulation does not operate automatically, but "at the discretion of the Executive Committee"—in most cases, on the application of the individual members concerned.

Members deceased: The Secretary presented the following statement of the Executive Committee:

The Association has suffered very great loss through the death during the year of eight members:

MAUDE E. S. ABBOTT, born in 1869, died September 2, 1940. Elected to membership in 1921. A member of the medical faculty of McGill University; editor of various medical journals; stimulator of interest in medical museums; contributor to and recognized authority on knowledge of congenital cardiac disease.

WAYNE JASON ATWELL, born October 19, 1889, died March 27, 1941. Elected to membership in 1914. Professor of Anatomy, University of Buffalo School of Medicine. Member of the Executive Committee 1937 to time of death. (A resolution on the death of Doctor Atwell has been adopted at this meeting and is printed on pages 1 to 3 of these Proceedings.)

ALEXANDER S. BEGG, born May 28, 1881, died September 26, 1940. Elected to membership in 1910. Waterhouse Professor of Anatomy and Dean, Boston University School of Medicine. Career in Anatomy divided between Drake University, Harvard Medical School and Boston University. Anatomical contributions on development of veins, cytology of intestinal epithelium, and anatomical anomalies.

WILLIAM BROWNING, born July 7, 1855, died January 5, 1941. His name is in the first published list of members (1890). Associated for 57 years with The Long Island College of Medicine as Lecturer on Anatomy and Physiology of the Nervous System; later as Neurologist. Author, among others,

of "The Normal and Pathological Circulation in the Central Nervous System". A distinguished Neurologist.

JOHN CREAN CARDWELL, born in 1876, died August 3, 1940. Elected to membership in 1917. Emeritus Professor of Physiology, The Long Island College of Medicine, with which institution he had been associated for 40 years, as a teacher of physiology and pharmacology.

HARRY BURR FERRIS, born May 21, 1865, died October 12, 1940. Elected to membership in 1894. Hunt Professor of Anatomy, Yale University. Greatly beloved teacher of anatomy at Yale for 42 years; made important contributions to anthropology.

GLADWYN KINGSLEY NOBLE, born September 20, 1894, died December 9, 1940. Elected to membership in 1923. Curator of the Museum of Natural History of New York and creator of the Department of Experimental Biology at the Museum. Published many valuable articles in herpetology and phylogeny, as well as on endocrinology and experimental morphology.

WILLIAM REES BREBNER ROBERTSON, born 1881, died March 15, 1941. Elected to membership in 1933. Assistant Professor of Histology, University of Iowa. Chief contributions were in the field of Genetics.

Memorial to Professor Adolphe Nicolas: Word of the death on May 9, 1939, of Professor Adolphe Nicolas, the last survivor of a distinguished list of honorary members of the Association, reached former President Ranson shortly before the 1940 meeting. In response to President Ranson's request, Dr. A. Weber, a pupil of Professor Nicolas, prepared a memorial, which, with the endorsement of the Association, appears below.

ADOLPHE NICOLAS

1861-1939

Professor Adolphe Nicolas, honorary member of the American Association of Anatomists, died at Nancy (France) on the 9th of May 1939. He was born on the 1st of March 1861, in the little Lorraine town of Pont-à-Mousson, which was famous in the 18th century for its University. He studied at the Faculty

of Medicine of Nancy, where he became successively "aide d'anatomie, prosecteur, chef des travaux, professeur agrégé" and in 1893 "professeur" of Anatomy. Through his successful teaching he was called in 1907 to the University of Paris. He took his retreat and left the capital in 1927 to retire to Nancy with his family, near his old and dear friends.

Professor A. Nicolas followed researches during all his long career, but he published relatively little, fearing always that his preparations were not perfect enough and his statements not sufficiently supported; he refused thus by excessive scrupulousness to make known very interesting observations on gastrulation in Reptiles. Also, when he agreed to present to a Congress a communication or a demonstration, he obtained the most complete conviction, as I heard Professor W. His, Sr. tell him at the meeting of the "Anatomische Gesellschaft" at Heidelberg in 1903.

Among A. Nicolas' most important publications can be quoted an histo-physiological work on the epithelial coating of the small intestine, especially during the absorption of fat (1890); a memoir on the elements of primitive kidney canalicules in Mammals (1891); researches on fecundation and segmentation in Reptiles ('03), pursued thanks to the Elizabeth Thompson Foundation; the study on the development of pancreas, liver and spleen in *Acipenser ruthenus* ('03). Also he issued, first with Charpy, then alone, the successive editions of Poirier's "Traité d'Anatomie humaine", many chapters of which he had written.

His competency was extended as well to macroscopic anatomy, as to cytology and comparative embryology. In France he was really a pioneer. Better than Ph. C. Sappey or Mathias Duval, he showed in his teaching and in all his publications the necessity of a widely extended culture, not confining human anatomy to the sole role of servant of surgery, but assigning to it its true place in general morphology. He has not been as well understood in Paris as in Lorraine, but his influence has, however, been very considerable. Thanks to it, he was able to found in 1899, with his friend E. Laguesse the "Association des Anatomistes". As long as he was the general secretary

of it, he grouped around him ardent workers and made a vocation for many disinterested searchers.

He had assisted, in his natal town, in August 1870, at a fight, prelude of big battles fought around Metz. The memory of that invasion has made his patriotism sometimes suspected, but more than anyone he thought that science should constitute the best of international bonds.

If the "Bibliographie anatomique" he had founded in 1892, and which lived until 1918, announced only works published in the French language, that periodical was widely open to works of scholars of all nations. It is also at Professor A. Nicolas' instigation that the "Federation internationale des Anatomistes", to which belongs the American Association, was founded in 1905. In 1908 he accepted the chairmanship, in Berlin, of the 22d meeting of the "Anatomische Gesellschaft."

The scientist in A. Nicolas was sometimes paralysed by a critical spirit, rejecting often a little hastily new theories or great hypotheses of work, to attach himself only to facts, which alone are indisputable; but the man was charming, modest and affectionate. His death was for his pupils, as well as for anatomy, an irreparable loss.

(Signed) A. WEBER (Geneva).

Members resigned:

Margaret Shea Gilbert, Appleton, Wis.
Theophilus S. Painter, Austin, Tex.

Members dropped for non-payment of dues for 1939 and 1940:

A. W. Kozelka, Two Rivers, Wis.
S. S. Schochet, Chicago, Ill.
E. M. Shearer, New York City

(The Constitution, in Article VI, Section I, provides that former members "may be reinstated at the discretion of the Executive Committee on payment of arrears".)

Number of members: The Secretary presented the following statistics on membership:

Number of members March 21, 1940	689
Deceased 8	
Resigned 2	
Dropped 3	13
	676
New members elected April 10, 1941	44
Number of members April 11, 1941	720

The Secretary's report on membership was then adopted by the Association.

Payment to Local Committee: The Secretary called attention to the fact that since 1929, when the Association raised the amount allotted to the Local Committee from \$100 to \$150, the number of members had increased from 472 to 689, with a corresponding increase in attendance at the meetings, and in the cost of entertainment. With this in mind, it was moved, seconded and passed that the Treasurer be authorized to pay the sum of \$200 from the general funds of the Association to the local committee on arrangements, to aid in defraying the cost of entertainment of the Association during the present meeting, and that steps be taken to make possible a further increase at future meetings.

Dues for 1941: The Secretary-Treasurer reported that the Executive Committee recommends that the dues for 1941 be raised to \$2.00, with the understanding that each member continue to receive automatically a copy of the "Abstracts", as during the current year; that in addition each member shall receive a copy of the "Proceedings" and "list of members" (the copies received by members in 1940 having been a gift of The Wistar Institute); and that the Association be prepared to increase the amount appropriated to the local committee on arrangements to \$250.

A motion was made, seconded and passed unanimously that the dues for 1941 be \$2.00.

Dues of members in countries of Europe and Asia: The Secretary reported the following: The Executive Committee recommends to the Association that members in belligerent and occupied countries of Europe and Asia be continued as members without payment of dues for the duration of hostilities, at the discretion of the Secretary-Treasurer. On motion made, seconded and passed this recommendation was adopted by the Association. It was pointed out that the number of members affected was twenty-seven.

Contribution to the Union of American Biological Societies: A motion was duly moved, seconded and passed that the Association appropriate the sum of \$10.00 as a contribution to the Union of American Biological Societies—such a contribution

having been made regularly every 3 years for a considerable period.

Place of next meeting: The Secretary reported the following action: The Executive Committee has decided to accept the invitation of Professor J. C. Hinsey of the Cornell University Medical College, New York City, to hold the 1942 meeting in New York under the auspices of Cornell University, on the Wednesday, Thursday and Friday, April 1, 2 and 3, in the week preceding Easter, which falls on April 5 in 1942.

It was further voted by the Executive Committee that the invitation of President F. Cyril James and Professor Cecil P. Martin of McGill University, Montreal, be given serious consideration for the 1943 meeting. Easter, in 1943, falls on April 25.

The Committee expresses its sincerest appreciation of several other cordial invitations which have been received for the 1942 meeting.

The American Journal of Anatomy: The Secretary reported for the Executive Committee that the President had received from Professor George W. Corner a letter of resignation from the managing editorship of The American Journal of Anatomy, to take effect in April, 1941; that the resignation had been accepted with very great regret; that, on recommendation of a special committee consisting of Warren H. Lewis, Chairman, Stacy R. Guild and George W. Bartelmez, Professor Philip E. Smith had been elected to the position of Managing Editor of The American Journal of Anatomy, subject to the endorsement of The Wistar Institute.

The Association then adopted unanimously the following expression of appreciation of the services of George W. Corner as Managing Editor of The American Journal of Anatomy:

In 1939, George W. Corner was selected by the American Association of Anatomists, with the endorsement of The Wistar Institute of Anatomy and Biology, to serve as Managing Editor of The American Journal of Anatomy. Dr. Corner has served with distinction. His editorship has been characterized by courtesy, a scholarly and scientific judgment and a

spirit of helpfulness. For this important service, the Association expresses and places on record its appreciation and thanks.

The Anatomical Record: The Secretary announced that Dr. E. A. Boyden, Managing Editor of The Anatomical Record, had reported to the Executive Committee that he had appointed as Associate Editors of The Anatomical Record, in place of Philip E. Smith and B. C. H. Harvey, resigned, J. C. B. Grant and William U. Gardner.

Relation of the Association to the journals: For the benefit of new members the Secretary pointed out that The American Journal of Anatomy and The Anatomical Record, while owned by The Wistar Institute of Anatomy and Biology, are sponsored by the American Association of Anatomists, which through its Executive Committee selects their Managing Editors, subject to the approval of The Wistar Institute; that members are granted a reduction of 25% from the regular subscription rates, not only of these two journals, but also of several other Wistar journals, and that each member who subscribes to one or more of the Wistar journals helps to strengthen this important phase of anatomical research.

Report on replies to questions sent to members of the American Association of Anatomists, May 1940: With the authorization of the Executive Committee the Secretary sent to all members in April, 1940, a set of questions pertaining to the experimental program arrangement of the Louisville meeting. Tabulation of the replies is as follows:

Number of members in Association	686		
Total replies received	307	45%	
Number of members attending Louisville meeting	252		
Replies received from members attending Louisville meeting	204	82%	
<i>Questions</i>			
1. Prefer for meeting Wed., Thurs., Fri.	213		
" " " Thurs., Fri., Sat.	79		
		<i>Yes</i>	<i>No</i>
2. Approve Wed. morning combined session	258	27	
3. (a) Approve 2: 00-3: 30 sessions for papers Wed. and Thurs.	235	17	
(b) Approve 3: 30-5: 30 session for demonstrations Wed. and Thurs.	238	10	

4. Approve treating motion pictures as "papers" requiring 16 mm. films, 12 minute time allowance	186	40
Qualified Yes	60	
5. Favor continuation of rule restricting to one the number of times a name may appear in each of three categories "paper from platform", "read by title", "demonstration" (Question 5 evidently misinterpreted for:)	246	49
(a) Favor more liberality in permitting demonstrations	174	90
(b) Favor allowing presentation of one "paper from platform" and introduction of one other paper from platform	207	81
(c) Favor more rigid restrictions on number of papers	110	123
Favor more rigid restrictions on quality of papers	128	80
6. Approve Friday afternoon invitation symposium	240	32
7. Favor additional symposia	87	161

Committee to cooperate with The Wistar Institute's "sponsorship of motion pictures" plan: The Secretary reported that the President had appointed a special Motion Picture Committee of three, consisting of E. R. Clark, Chairman, W. H. Lewis and C. C. Speidel for 1940-41 and that the Committee had been reappointed for 1941-42. The Committee made the following report:

The Committee, along with a similar Committee representing the American Society of Zoologists, has cooperated fully with The Wistar Institute, in connection with the working out of details and the starting in operation of the plan to create, at The Wistar Institute, a depository for motion picture films of biological interest, designed both to preserve valuable records and to make them available for research studies, and perhaps also for distribution (rental and sale). Persons having valuable motion picture films of biological interest may send copies to The Wistar Institute. The Institute, through its Advisory Committee, inspects the films and endorses or rejects them. If a film is endorsed, a brief review of the content and character will be published in *The Anatomical Record* (the first 2 pages of reviews have been published in the March, 1941, number of *The Anatomical Record*) and the owner may deposit a copy with The Wistar Institute, under various options: (a) it may remain at the Wistar to be consulted as research material by qualified investigators; (b) a duplicate copy may be available for renting; (c) arrangements may be made for the sale of copies.

In addition to several consultations with Dr. Farris, Executive Director of The Wistar Institute, regarding details of the plan, members of your Committee have viewed—and written reviews of—over thirty films.

It is the opinion of the Committee that this venture on the part of The Wistar Institute, which is entirely non-profit to the Institute, bids fair to render valuable aid to both research and teaching in Anatomy, and that a committee of three should be continued.

(Signed) W. H. LEWIS
C. C. SPEIDEL,
E. R. CLARK, Chairman

Appointed Representatives: It was announced that the President had appointed as representatives of the Association to the Second Pan American Congress of Endocrinology, Montevideo, March 5-8, 1941, G. W. Corner, H. M. Evans and R. L. Zwemer; as delegate to The University of Chicago 50th Anniversary Celebration September 27, 28 and 29, 1941, S. W. Ranson; and as delegate to the 175th Anniversary of the Founding of Rutgers College, October 9 and 10, 1941, C. F. W. McClure.

Shortage of dissecting material: The Committee appointed at the Fifty-sixth Session to investigate certain possibilities regarding the shortage of dissecting material reported informally that it had refrained from activities on finding the following resolution of the American Medical Association, which to a certain extent answered Professor Danforth's question:

"Resolved, that those cases removed from the jurisdiction of a hospital by coroner or medical examiner, and in consequence not available as teaching material for interns, may be deducted from the total hospital deaths in computing autopsy percentages. This provision also extends to bodies legally assigned to qualified educational institutions for dissection."

Furthermore, the American Medical Association is carrying out an extensive survey of the adequacy of dissecting material, and it was decided that the Association should await the results of this investigation.

Report of the Committee on Anatomical Nomenclature: The Chairman of this Committee, Professor C. M. Jackson, submitted the following report:

At the Toronto meeting of our Association (see Proceedings, Anatomical Record, 1937, vol. 68, Supplement, pp. 6-7) it was reported that the International Commission on Anatomical Nomenclature had invited us to participate in the establishment of an international nomenclature through a revision of the Nomina Anatomica. After a discussion of this proposal, the Association voted to authorize our American committee to formulate desirable changes in nomenclature and to submit them to the International Commission for consideration.

Our committee accordingly formulated a report which was sent to the International Commission on July 28, 1937. This extensive report (57 typewritten pages) summarized four years of work by our committee, listing numerous proposed changes in nomenclature together with the reasons therefore. In acknowledging

receipt of this report, Professor Stieve (Chairman of the Commission) stated that it was thorough and would doubtless be very helpful to the Commission in connection with the changes proposed by the various other countries. Professor Stieve later wrote that most of the countries, aside from the American and British, appeared willing to accept the NA (JNA) terminology, with comparatively few changes. Further consideration of the subject has been interrupted by the war, however, so that no final decision by the Commission can be expected in the immediate future.

(Signed) C. M. JACKSON
Chairman of Committee

April 7, 1941.

National Defense Committee: It was reported by the Secretary that the Executive Committee had chosen a special National Defense Committee, consisting of J. S. Nicholas, Chairman, with the President and Secretary-Treasurer ex officio members, which should hold itself in readiness to organize anatomical projects which might be requested by the National Government, or the National Research Council, in connection with National Defense. It was explained that the three appointees had already been serving as representatives of the Association on the National Resources Planning Board. As Executive Secretary, Dr. J. S. Nicholas was reported to have played an important part in assembling data regarding Anatomists in the preparation of a national roster of scientific and specialized personnel.

In this connection mention was made of the fact that Professor Ross G. Harrison is Chairman of the National Research Council, and that Professor Lewis H. Weed is Chairman of the Division of Medical Sciences of the National Research Council—both performing active and invaluable services in the National Defense Program.

The main Business Session adjourned at 1:05 P.M.

FRIDAY, APRIL 11, 1941

12:45 p.m. Concluding Business Session, President Smith in the chair.

Resolution of Appreciation: The following resolution, proposed by Professor W. H. F. Addison, was unanimously adopted:

It is now our pleasant duty as guests of the University to express our feelings of appreciation for all that has been done for us, both in preparation for and during these meetings.

At this, the closing business meeting of the Fifty-seventh Session of the American Association of Anatomists, we extend our sincere thanks to our hosts of The University of Chicago, and especially the Local Committee, for their painstaking preparation, their hearty welcome and their gracious entertainment.

We hope that they will share with us many pleasant memories of this session, and we wish to add our best wishes for the University's continued progress during its second fifty years of service, now about to begin.

The concluding Business Session then adjourned.

ELIOT R. CLARK

Secretary of the Fifty-seventh Session of
the American Association of Anatomists

ERRATUM

In abstract no. 48 entitled: "The connective tissue of placentomata in rats, etc.", by J. M. Wolfe and A. M. Wright, in *The Anatomical Record*, vol. 79, Supplement no. 2, Mar. 25, 1941, p. 65, the eleventh line should read:

"The fibrillar connective tissue ranged from fine fibrils to thick fibers (up to 3μ);"

AMERICAN ASSOCIATION OF ANATOMISTS

CONSTITUTION

(AS AMENDED MARCH 31, 1940)

ARTICLE I

Section 1. The name of the society shall be "The American Association of Anatomists."

Sec. 2. The purpose of the Association shall be the advancement of anatomical science.

ARTICLE II

Section 1. The officers of the Association shall consist of a President, a First Vice-President, a Second Vice-President, and a Secretary who shall also act as Treasurer. The president and the two Vice-Presidents shall be elected for two years, the Secretary for four years. In case of absence of the President and one or both Vice-Presidents, the members of the Executive Committee, in order of seniority, shall preside. The election of all the officers shall be by ballot at the annual meeting of the Association and their official term shall commence with the close of the annual meeting.

Sec. 2. At the annual meeting next preceding an election, the President shall name a nominating committee of three members. This committee shall make its nominations to the Secretary not less than two months before the annual meeting at which the election is to take place. It shall be the duty of the Secretary to mail the list to all members of the Association at least one month before the annual meeting. Additional names for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

ARTICLE III

The management of the affairs of the Association shall be delegated to an Executive Committee, consisting of twelve members, including the officers. Two members of the Executive Committee shall be elected annually, and, so far as possible, election of members of the Executive Committee shall be in proportion to the geographical distribution of members. Five shall constitute a quorum of the Executive Committee.

ARTICLE IV

The Association shall meet at least annually, the time and place to be determined by the Executive Committee. The annual meeting for the election of officers shall be the meeting of convocation week, or in case this is not held, the first meeting after the new year.

ARTICLE V

Section 1. Candidates for membership must be persons engaged in the investigation of anatomical or cognate sciences, and shall be proposed in writing to the Executive Committee by two members, who shall accompany the recommendation by a list of the candidate's publications, together with references. Their election by the Executive Committee, to be effective, shall be ratified by the Association in open meeting.

Sec. 2. Honorary members may be elected from those who have distinguished themselves in anatomical research. Nominations by the Executive Committee must be unanimous and their proposal with a reason for recommendations shall be presented to the Association at an annual meeting, a three-fourths vote of members present being necessary for an election.

ARTICLE VI

Section 1. The annual dues of the Association shall be determined at each annual meeting for the ensuing year. A member in arrears for dues for two years shall be dropped by the Secretary at the next meeting of the Association, but may be reinstated at the discretion of the Executive Committee on payment of arrears.

* *Sec. 2.* Any member who has paid the annual dues for thirty years, or who has attained the age of sixty-five years, or who has retired because of illness, may be relieved of the payment of the annual dues at the discretion of the Executive Committee.

ARTICLE VII

Section 1. Twenty members shall constitute a quorum for the transaction of business.

Sec. 2. Any change in the constitution of the Association must be presented in writing at one annual meeting in order to receive consideration and be acted upon at the next annual meeting; due notice of the proposed change to be sent to each member at least one month in advance of the meeting at which such action is to be taken.

Sec. 3. The ruling of the Chairman shall be in accordance with "Robert's Rules of Order."

* Article VI, Sec. 2, was adopted as an amendment at the 56th Session, March 21, 1940.

ORDERS

The following orders have been adopted by this Association:

Newly elected members must qualify by payment of dues for one year within thirty days after election.

The maximum limit of time for the reading of papers shall be twelve minutes. (Altered to ten minutes for the 1938 Session.)

The Secretary and Treasurer shall be allowed his traveling expenses and the sum of \$10 toward the payment of his hotel bill, at each session of the Association.

The Executive Committee shall nominate each year, for election by the Association at the Annual Meeting, two (or three) members to serve as representatives to the Council of the American Association for the Advancement of Science at the meetings of the Council during the same year.

The Executive Committee shall nominate from time to time, for election by the Association, one member to serve as representative to the Union of Biological Societies, and one as representative to the National Research Council; their terms of office to be three years.

The Executive Committee shall nominate each year, for election by the Association, one member to serve as representative on the Committee for Standardization of Biological Stains, the same person to be reappointed from year to year as the Association sees fit.

AMERICAN ASSOCIATION OF ANATOMISTS
OFFICERS AND LIST OF MEMBERS

Officers

<i>President</i>	Philip E. Smith
<i>First Vice-President</i>	Carl G. Hartman
<i>Second Vice-President</i>	Davenport Hooker
<i>Secretary-Treasurer</i>	Eliot R. Clark

Executive Committee

For term expiring 1942.....	George W. Corner, Olof Larsell
For term expiring 1943.....	Stacy R. Guild, Edward A. Boyden
For term expiring 1944.....	Joseph C. Hinsey, Carl C. Speidel
For term expiring 1945.....	E. Horne Craigie, Walter E. Sullivan

Delegates to the Council of A.A.A.S. for 1941

F. H. Swett
J. T. Patterson
G. W. Bartelmez

Representative to the National Research Council 1940-1943

Eliot R. Clark

Member of the Commission on Standardization of Biological Stains

Harold A. Davenport

Delegate of Union of Biological Societies, 1941-1944

Stacy R. Guild

Committee on Nominations for 1942

Edward A. Boyden, *Chairman*

Carl G. Hartman Joseph C. Hinsey

Committee on Anatomical Nomenclature

Clarence M. Jackson, *Chairman*

Stephen W. Ranson Robert J. Terry

George W. Corner, *Secretary*

Committee on National Defense

J. S. Nicholas, *Chairman*

The President and Secretary-Treasurer, ex officio

Motion Picture Committee

E. R. Clark, *Chairman*

Warren H. Lewis Carl C. Speidel

MEMBERS

- ABELL, RICHARD G., Ph.D., Instructor in Anatomy, *University of Pennsylvania School of Medicine, Philadelphia, Pa.*
- ABERCROMBIE, WARREN F., A.B., Sc.M., Ph.D., Associate Professor of Biology, *Howard College, Birmingham, Ala.*
- ABERLE, (MRS.) SOPHIE B. D., A.B., A.M., Ph.D., M.D., General Superintendent of Pueblo Agency, Albuquerque; Research Associate, Carnegie Institution of Washington; *United Pueblos Agency, Box 1346, Albuquerque, New Mexico.*
- ADAMS, A. ELIZABETH, A.B., A.M., Ph.D., Professor of Zoology, *Mount Holyoke College, South Hadley, Mass.*
- ADAMS, L. A., A.B., A.M., Ph.D., Professor of Zoology, Curator of Museum of Natural History, University of Illinois, *401 Vermont St., Urbana, Ill.*
- ADAMSTONE, F. B., B.A., M.A., Ph.D., Associate Professor of Zoology, *University of Illinois, Urbana, Ill.*
- ADDISON, WILLIAM HENRY FITZGERALD, B.A., M.D., Professor of Normal Histology and Embryology, *School of Medicine, University of Pennsylvania, Philadelphia, Pa.*
- ADELMANN, HOWARD B., B.A., M.A., Ph.D., Professor of Histology and Embryology, *Cornell University, Stimson Hall, Ithaca, N. Y.*
- ALEXANDER, WILLIAM F., B.S., M.S., Ph.D., Instructor in Anatomy, *Louisiana State University, School of Medicine, New Orleans, La.*
- ALLEN, BENNET MILLS, Ph.D., Professor of Zoology, University of California at Los Angeles, *909 Micheltorena St., Los Angeles, Calif.*
- ALLEN, EDGAR, A.M., Ph.D., Sc.D., (Vice-Pres. '30-'32), Professor of Anatomy, Yale University School of Medicine, *333 Cedar St., New Haven, Conn.*
- ALLEN, EZRA, A.M., Ph.D., Retired. *R.D. 2, Box 91A, De Land, Fla.*
- ALLEN, LANE, M.S., Ph.D., M.D., Assistant Professor of Anatomy, *University of Georgia School of Medicine, Augusta, Ga.*
- ALLEN, WILLARD M., B.S., M.S., M.D., *Washington University, School of Medicine, St. Louis, Mo.*
- ALLEN, WILLIAM F., A.M., Ph.D., Head of Department of Anatomy, *University of Oregon Medical School, Portland, Ore.*
- ALLIS, EDWARD PHELPS, JR., C.E., M.D., LL.D. (Charter member), *Palais de Carnoles, Menton (A.M.), France.*
- ALTSCHUL, RUDOLF, M.D., Instructor in Anatomy, *University of Saskatchewan, Saskatoon, Canada.*
- ANDERSEN, DOROTHY H., Associate in Pathology, Columbia University; Assistant Pathologist, *Babies' Hospital, 167th St. and Broadway, New York City.*
- ANDREW, WARREN, M.S., Ph.D., Assistant Professor in Histology and Embryology, *Department of Anatomy, Baylor University, College of Medicine, Dallas, Tex.*
- ANGULO Y GONZALEZ, ARMANDO W., B.L., B.S., A.B., A.M., Ph.D., *418 Beverly Blvd., Upper Darby, Pa.*
- ANSON, BARRY J., A.B., A.M., Ph.D., Associate Professor of Anatomy, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*

- APGAR, BESSIE D., Ph.D., *R.D. 1, Old Union Deposit Rd., Harrisburg, Pa.*
- APGAR, CHARLES S., JR., Ph.D., *R.D. 1, Old Union Deposit Rd., Harrisburg, Pa.*
- APPLEBY, J. I., A.B., M.B., A.M., M.D., Physician and Surgeon, Company Surgeon, N. Y. C. & St. L. R. R. Co., *105 1/2 East Main St., Bellevue, O.*
- AREY, LESLIE B., Ph.D., Sc.D., (Ex. Com. '30-'34), Robert Laughlin Rea Professor of Anatomy, and Chairman of the Division of Anatomy, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*
- ARMSTRONG, PHILIP BROWNELL, B.S., M.D., Professor of Anatomy, *College of Medicine, Syracuse University, Syracuse, N. Y.*
- ASDELL, SYDNEY ARTHUR, M.A., Ph.D., Professor of Animal Physiology, State College of Agriculture, *Cornell University, Ithaca, N. Y.*
- ASHLEY-MONTAGU, MONTAGUE FRANCIS, Ph.D., Associate Professor of Anatomy, *Hahnemann Medical School and Hospital, 235 North 15th St., Philadelphia, Pa.*
- ATTERBURY, RUTH RAND, A.M., Ph.D., *Kings' Point, Great Neck, L. I.*
- BADERTSCHER, JACOB A., Ph.D., Professor of Anatomy, Indiana University School of Medicine, *312 South Fess Ave., Bloomington, Ind.*
- BAGLEY, CHARLES, JR., M.D., Professor of Neurosurgery, University of Maryland Medical School; Associate in Experimental Neurology, Johns Hopkins University Medical School, *The Latrobe Apts., Baltimore, Md.*
- BAILEY, PERCIVAL, M.D., Ph.D., Professor of Neurology and Neurosurgery, *University of Illinois, 1853 W. Polk St., Chicago, Ill.*
- BAILEY, RALPH JORDAN, B.S., M.S., Ph.D., Assistant Professor of Anatomy, *School of Medicine, University of Arkansas, Little Rock, Ark.*
- BAILLIF, R. N., Ph.D., Instructor in Anatomy, *Louisiana State University, Medical Center, New Orleans, La.*
- BAITSELL, GEORGE ALFRED, B.S., M.A., Ph.D., Professor of Biology, Yale University, *Osborn Zoological Laboratory, New Haven, Conn.*
- BAKER, DAN D., M.A., M.D., Assistant Professor of Anatomy, *Louisiana State University, Medical Center, New Orleans, La.*
- BAKER, R. C., A. B., M.A., Ph.D., Professor of Anatomy, *Department of Anatomy, Hamilton Hall, Ohio State University, Columbus, O.*
- BAKER, ROGER D., M.D., Assistant Professor of Pathology, *Duke University, Durham, N. C.*
- BALDWIN, WESLEY MANNING, A.B., A.M., M.D., *Box 441, York Beach, Me.*
- BAENARD, JOHN W., M.S., Ph.D., Assistant Professor of Anatomy, *Georgetown University, Washington, D. C.*
- BARRETT, W. C., JR., Ph.D., Assistant Professor of Histology and Embryology, *School of Medicine, Western Reserve University, Cleveland, O.*
- BARRIS, RALPH W., Ph.D., Assistant Professor of Anatomy, *George Washington University Medical School, Washington, D. C.*
- BARRON, DONALD H., B.A., M.S., Ph.D., Department of Zoology, *University of Missouri, Columbia, Mo.*
- BARTELMEZ, GEORGE W., Ph.D. (Vice-Pres. '32-'34, Exec. Com. '36-'40). Professor of Anatomy, *The University of Chicago, Chicago, Ill.*
- BARTSCH, PAUL, B.S., M.S., Ph.D., D.Sc., Curator of Mollusks and Cenozoic Invertebrates, *U. S. National Museum, Washington, D. C.*

- BAST, T. H., B.A., Ph.D., Professor of Anatomy, *University of Wisconsin, 303 Science Hall, Madison, Wis.*
- BATSON, O. V., A.M., M.D., Professor of Anatomy, *Department of Anatomy, Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pa.*
- BAUMGARTNER, WILLIAM J., A.B., A.M., Ph.D., Professor of Zoology, *University of Kansas, 1209 Ohio St., Lawrence, Kan.*
- BEAMS, HAROLD W., Ph.D., Professor of Zoology, *State University of Iowa, Iowa City, Ia.*
- BEAN, ROBERT BENNETT, B.S., V.P.I., M.D., Professor of Anatomy, *University of Virginia, Box 1536, University Station, Charlottesville, Va.*
- BEATON, LINDSAY E., A.B., B.M., M.D., M.S., National Research Council Fellow, 1940-41, *Northwestern University Medical School, Chicago, Ill.*
- BEATTIE, JOHN, M.D., D.Sc., Conservator of Museum and Director of Research, *Royal College of Surgeons; Colonel, Army Medical Service; Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2, England.*
- BECKER, ROLAND F., B.S., M.S., Ph.D., Instructor in Anatomy, *Northwestern University Medical School, Chicago, Ill.*
- BÉLANGER, LEONARD F., A.B., M.D., A.M., Assistant, *Department of Histology and Embryology, University of Montreal, Montreal, Canada.*
- BENJAMIN, JAMES W., A.B., M.S., Ph.D., Associate Professor of Histology, Embryology and Neuroanatomy, *New York Medical College, Flower and Fifth Avenue Hospitals, New York City.*
- BENNETT, GEORGE A., A.B., M.D., Assistant Professor of Anatomy, *Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia, Pa.*
- BENNETT, HENRY STANLEY, A.B., M.D., Instructor in Anatomy and Pharmacology, *Harvard Medical School, Boston, Mass.*
- BENOIT, JACQUES, M.D., Professeur d'Histologie, *Faculté de Médecine, Alger, France.*
- BENSLEY, ROBERT RUSSELL, A.B., M.B., Sc.D. (Second Vice-Pres. '06-'07, Ex. Com. '08-'12, President '18-'20), Professor Emeritus of Anatomy, *The University of Chicago, Chicago, Ill.*
- BENSLEY, SYLVIA H., M.D., Instructor in Anatomy, *Department of Anatomy, The University of Chicago, Chicago, Ill.*
- BEST, R. RUSSELL, B.Sc., M.D., Assistant Professor of Anatomy, Associate Professor of Surgery, *University of Nebraska, College of Medicine, 527 Medical Arts Building, Omaha, Neb.*
- BEVAN, ARTHUR DEAN, M.D. (Ex. Com. '96-'98), Professor of Surgery, *The University of Chicago, 122 South Michigan Blvd., Chicago, Ill.*
- BEVELANDER, GERRIT, Ph.D., Assistant Professor of Anatomy, *College of Dentistry, New York University, 477 First Ave., New York City.*
- BIGELOW, ROBERT P., S.B., Ph.D., Professor Emeritus of Zoology and Parasitology, *Massachusetts Institute of Technology, 72 Blake Rd., Brookline, Mass.*
- BISHOP, KATHERINE SCOTT, A.B., M.D., *1508 La Loma Ave., Berkeley, Calif.*
- BLAIR, DUNCAN M., M.B., Ch.B., D.Sc., Professor of Anatomy, *University of Glasgow, Glasgow, Scotland.*
- BLAIR, VILRAY PAPIN, M.D., A.B., Professor of Clinical Surgery, *Washington University School of Medicine, 400 Metropolitan Building, St. Louis, Mo.*

- BLAISDELL, FRANK ELLSWORTH, SR., M.D., Emeritus Professor of Surgery, Medical Department of Stanford University, *Lane Medical Library Building, corner Webster and Sacramento Sts., San Francisco, Calif.*
- BLINCOE, HOMER, A.B., M.S., M.D., Professor of Anatomy, *Emory University, Emory, Ga.*
- BLOOM, WILLIAM, A.B., M.D., Associate Professor of Anatomy, *Department of Anatomy, The University of Chicago, Chicago, Ill.*
- BLOUNT, ISABEL H., A.B., A.M., Ph.D., Voluntary Research Worker, *Department of Anatomy, University of Minnesota, Minneapolis, Minn.*
- BLOUNT, R. F., M.S., Ph.D., Assistant Professor of Anatomy, *University of Minnesota, Minneapolis, Minn.*
- BLUMENFELD, CHARLES M., Ph.D., M.D., Resident in Pathology, *City Hospital, Cleveland, O.*
- BODIAN, DAVID, S.B., Ph.D., M.D., Assistant Professor of Anatomy, *Western Reserve University Medical School, Cleveland, O.*
- VON BONIN, GERHARDT, M.D., Professor of Anatomy, *University of Illinois, College of Medicine, 1853 West Polk St., Chicago, Ill.*
- BOOK, M. H., M.D., Instructor in Anatomy, *Tufts College Medical School, 416 Huntington Ave., Boston, Mass.*
- BOWIE, DONALD J., B.Sc., Med., M.A., Ph.D., Assistant Professor of Anatomy, *Medical College, Winnipeg, Manitoba, Canada.*
- BOYD, EDITH, M.D., Assistant Professor of Anatomy, *University of Minnesota, Minneapolis, Minn.*
- BOYDEN, EDWARD ALLEN, A.M., Ph.D. (Ex. Com. '28-'32, '39-), Professor of Anatomy, *Institute of Anatomy, University of Minnesota, Minneapolis, Minn.*
- BOYER, ESTHER L., M.A., Ph.D., M.D., Instructor in Anatomy, *Woman's Medical College of Pennsylvania, Philadelphia, Pa.*
- BRADBURY, JAMES T., B.S., M.S., Sc.D., Endocrinologist, *Bureau of Dairy Industry, U. S. Department of Agriculture, Beltsville, Md.*
- BRASHEAR, ALTON D., M.S., D.D.S., Assistant Professor of Anatomy, *Medical College of Virginia, Richmond, Va.*
- BREMER, JOHN LEWIS, A.B., M.D. (Ex. Com. '15-'18, '33-'37), Hersey Professor of Anatomy, *Harvard Medical School, Boston, Mass.*
- BREWER, JOHN I., M.D., Ph.D., Associate in Gynecology, *Northwestern University Medical School and St. Luke's Hospital, Chicago, 104 S. Michigan, Chicago, Ill.*
- BROWMAN, LUDVIG G., Ph.D., Assistant Professor in Zoology and Physiology, *Montana State University, Missoula, Mont.*
- BRUES, AUSTIN MOORE, A.B., M.D., Associate Physician, *Huntington Memorial Hospital, Boston, Mass.*
- BRYCE, THOMAS H., M.A., M.D., F.R.S., Emeritus Professor of Anatomy, *University of Glasgow, The Loaning, Peebles, Scotland.*
- BUCHANAN, A. R., B.S., M.S., M.D., Associate Professor of Anatomy, *University of Colorado Medical School, 4200 E. Ninth Ave., Denver, Colo.*
- BULLARD, H. HAYS, A.M., Ph.D., M.D., Pathologist and Director of Laboratories, *St. Vincent's Hospital, Erie, Pa.*
- BUNTING, CHARLES HENRY, B.S., M.D., Professor of Pathology, *University of Wisconsin, Service Memorial Institute, Madison, Wis.*

- BURNS, B. I., A.B., A.M., Ph.D., M.D., Professor of Anatomy; Dean, School of Medicine, *Louisiana State University, 1542 Tulane Ave., New Orleans, La.*
- BURNS, ROBERT K., JR., A.B., Ph.D., Research Associate, *Carnegie Laboratory of Embryology, Wolfe and Madison Sts., Baltimore, Md.*
- BURR, HAROLD SAXTON, Ph.D., (Ex. Com. '29-'33), E. K. Hunt Professor of Anatomy, Head of Section of Neuro-Anatomy, School of Medicine, Yale University, *333 Cedar St., New Haven, Conn.*
- BURROWS, MONTROSE T., A.B., M.D., Physician and Surgeon, *201 North El Malino Ave., Pasadena, Calif.*
- BUTCHER, EARL O., A.M., Ph.D., Professor of Biology, *Hamilton College, Griffin Road, College Hill, Clinton, N. Y.*
- BUTLER, ELMER G., A.B., Ph.D., Professor of Zoology, *Laboratory of Comparative Anatomy, Princeton University, Princeton, N. J.*
- BUYSE, ADRIAN, A.B., Ph.D., Assistant Professor of Zoology, *The University of Rochester, Rochester, N. Y.*
- CALKINS, LEROY A., M.D., M.S., Ph.D., Professor of Obstetrics and Gynecology, *University of Kansas Hospital, Kansas City, Kan.*
- CAMERON, ANGUS L., A.B., M.S., M.D., Ph.D., *Northwest Clinic, Minot, N. Dak.*
- CAMERON, JOHN A., A.B., A.M., Ph.D., Assistant Professor of Anatomy, *University of Missouri, Columbia, Mo.*
- CAMPBELL, BERRY, A.B., Ph.D., Fellow of the John Simon Guggenheim Foundation, *Department of Physiology, Rockefeller Institute for Medical Research, New York City.*
- CANDELA, POMPEO B., M.D., Research Associate in Anatomy, *New York Medical College, New York City.*
- CAREY, EBEN J., M.S., Sc.D., M.D., Dean and Professor of Anatomy and Director of Anatomy Department, *Marquette University School of Medicine, 6119 W. Wisconsin Ave., Wauwatosa, Wis.*
- CARPENTER, RUSSELL L., B.S., Ph.D., Professor of Zoology, *Tufts College, Medford, Mass.*
- CARVER, GAIL L., A.B., A.M., Professor of Biology, *Mercer University, Macon, Ga.*
- CASAMAJOR, LOUIS, A.M., M.D., Professor of Clinical Neurology, *Columbia University, 706 West 168th St., New York City.*
- CASEY, ALBERT E., M.D., Assistant Professor of Pathology and Bacteriology, *Louisiana State University School of Medicine, New Orleans, La.*
- CHAMBERS, ROBERT, JR., A.M., Ph.D. (Second Vice-Pres. '39-'40), Research Professor of Biology, *New York University, New York City.*
- CHANDLER, SIMON B., A.B., A.M., M.D., Professor of Anatomy, *West Virginia University School of Medicine, Morgantown, W. Va.*
- CHANG, CHUN, M.D., Associate Professor of Anatomy, *National Medical College of Shanghai, 373 Ave. Haig, Shanghai, China.*
- CHARIPPER, HARRY A., B.S., M.S., Ph.D., Professor of Biology (Tissue Cytology, Endocrinology, Histology and Comparative Haematology), Chairman of Department, *Washington Square College, New York University, New York City.*
- CHARLES, CECIL M., A.B., M.A., Ph.D., M.D., Assistant Professor of Anatomy, *Washington University School of Medicine, St. Louis, Mo.*
- CHASE, RALPH E., A.B., B.Sc., A.M., Instructor in Anatomy, *Oklahoma University Medical School, Oklahoma City, Okla.*

- CHASE, SAMUEL WOOD, A.B., A.M., Ph.D., Associate Professor of Histology and Embryology, *Western Reserve University, 2109 Adelbert Road, Cleveland, O.*
- CHERNER, MAXWELL, M.D., Demonstrator of Anatomy, *Jefferson Medical College, Philadelphia, Pa.*
- CHILD, CHARLES MANNING, M.S., Ph.D., D.Sc., Professor Emeritus of Zoology, *Jordan Hall, Stanford University, Calif.*
- CHOUKE, KEHAR S., M.A., M.D., Assistant Professor of Anatomy, *Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pa.*
- CHRISTENSEN, KERMIT, Ph.D., Assistant Professor of Microanatomy, *St. Louis University School of Medicine, 1402 South Grand Blvd., St. Louis, Mo.*
- CLARK, ELEANOR LINTON, A.M., Voluntary Research Worker, Department of Anatomy, *Medical Department, University of Pennsylvania, Philadelphia, Pa.*
- CLARK, ELIOT R., A.B., M.D., Sc.D. (Ex. Com. '16-'19, Sec.-Treas. '38-), Professor of Anatomy and Director of Department of Anatomy, *Medical Department, University of Pennsylvania, Philadelphia, Pa.*
- CLARK, GEORGE, B.S., M.S., Ph.D., Instructor in Neurology, *Medical College of the State of South Carolina, Charleston, S. C.*
- CLARK, SAM L., M.S., Ph.D., M.D., Professor of Anatomy, *Vanderbilt University School of Medicine, Nashville, Tenn.*
- CLAUSEN, HARRY J., A.B., M.S., Ph.D., Instructor in Anatomy, *University of Colorado School of Medicine, Denver, Colo.*
- CLEMENTS, LEO, Ph.D., Associate Professor of Anatomy, Member Nebraska Basic Science Board, *Creighton University Medical School, Omaha, Neb.*
- CLEVELAND, RUCKER, Ph.D., Assistant in Anatomy, *Vanderbilt University School of Medicine, Nashville, Tenn.*
- COBB, W. MONTAGUE, M.D., Ph.D., Associate Professor of Anatomy, *Howard University, Washington, D. C.*
- COE, WESLEY R., Ph.D., Professor of Biology Emeritus, *Yale University, Osborn Zoological Laboratory, New Haven, Conn.*
- COGHILL, GEORGE E., A.B., M.S., Ph.D., Sc.D. (Ex. Com. '27-'31, Pres. '32-'34), *R.F.D. 2, Box 77 A, Gainesville, Fla.*
- COHN, ALFRED E., A.B., M.D., Member, *Rockefeller Institute for Medical Research, New York City, N. Y.*
- COLE, ARCH EVAN, A.B., Ph.D., Assistant Professor, Anatomy Department, *University of Louisville, Louisville, Ky.*
- COLE, ELBERT C., Ph.D., Professor of Biology, *Williams College, Williamstown, Mass.*
- COLE, HAROLD H., Ph.D., Associate Professor of Animal Husbandry, University of California, and Associate Animal Husbandman, *Experiment Station of the College of Agriculture, Davis, Calif.*
- COLLINGS, M. RAYMOND, A.B., M.D., Professor of Anatomy, *Medical College, Wayne University, 1512 St. Antoine St., Detroit, Mich.*
- COMAN, DANA, A.B., A.M., M.D., Assistant in Psychiatry, *Johns Hopkins University, Johns Hopkins Medical School, Baltimore, Md.*
- CONEL, JESSE LEROY, A.M., Ph.D., Professor of Anatomy, *Boston University School of Medicine, 45 Arlington St., Newton, Mass.*
- CONGDON, EDGAR DAVIDSON, Ph.D., Professor of Anatomy, *Long Island College of Medicine, 350 Henry St., Brooklyn, N. Y.*

- CONKLIN, EDWIN GRANT, A.M., Ph.D., Sc.D., LL.D., Professor Emeritus of Biology, Princeton University, 139 Broadmead Ave., Princeton, N. J.
- COPENHAVER, WILFRED M., A.B., Ph.D., Associate Professor of Anatomy, College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.
- CORBIN, KENDALL B., B.A., M.D., Professor of Microscopic Anatomy, University of Tennessee College of Medicine, Memphis, Tenn.
- COREY, EDWARD LYMAN, A.B., Ph.D., Assistant Professor of Physiology, University of Virginia School of Medicine, University, Va.
- CORNER, GEORGE W., A.B., M.D. (Ex. Com. '26-'30, '38-; Sec.-Treas. '30-'38), Director, Department of Embryology, Carnegie Institution of Washington, Wolfe and Madison Sts., Baltimore, Md.
- CORNING, H. K., M.D., Professor of Anatomy (retired), 1107 Fifth Ave., New York City.
- COUNT, EARL W., Ph.D., Assistant Professor of Anatomy, New York Medical College, Flower and Fifth Avenue Hospitals, New York City.
- COVELL, W. P., M.S., Ph.D., M.D., Lecturer in Otology, Rhinology and Laryngology, University of California Medical Center; Associate Professor of Research Medicine, George Williams Hooper Foundation, San Francisco, Calif.
- COWDREY, EDMUND V., Ph.D. (Ex. Com. '26-'30, 1st Vice-Pres. '34-'36), Professor of Cytology, Washington University Medical School, St. Louis, Mo.
- CRAIGIE, E. HORNE, B.A., Ph.D. (Ex. Com. '41-), Associate Professor of Comparative Anatomy and Neurology, Department of Biology, University of Toronto, Toronto 5, Canada.
- CRILE, GEORGE, M.D., F.A.C.S., Professor Emeritus of Surgery, Western Reserve University; Director, Cleveland Clinic Foundation; Surgeon, Cleveland Clinic Hospital; Cleveland Clinic, 93rd and Euclid, Cleveland, O.
- CROSBY, ELIZABETH CAROLINE, M.S., Ph.D., Professor of Anatomy, University of Michigan, Department of Anatomy, University of Michigan, Ann Arbor, Mich.
- CROUCH, RICHARD L., Ph.D., M.D., Associate Professor of Anatomy, University of Missouri, Columbia, Mo.
- CUAJUNCO, FIDEL, M.D., Assistant Professor of Anatomy, College of Medicine, University of the Philippines, 620 Colorado St., Manila, P. I.
- CULLEN, THOMAS S., M.B., D.Sc., LL.D. (Tor.), Emeritus Professor of Gynecology, Johns Hopkins University, Visiting Gynecologist to the Johns Hopkins Hospital, 20 E. Eager St., Baltimore, Md.
- CUMMINS, HAROLD, AB., Ph.D. (Ex. Com. '36-'40), Professor and Head of Division of Microscopic Anatomy, Tulane University Medical School, Department of Anatomy, Sta. 20, New Orleans, La.
- CUNNINGHAM, ROBERT S., B.S., A.M., M.D., Sc.D. (Ex. Com. '32-'36), Professor of Anatomy, and Dean, Albany Medical College, Albany, N. Y.
- CURTIS, GEORGE M., A.M., Ph.D., M.D., Professor of Surgery and Chairman of the Department of Research Surgery, Kinsman Hall, Ohio State University, Columbus, O.
- CUTULY, EUGENE, Ph.D., Instructor in Anatomy, Wayne University College of Medicine, 1512 St. Antoine, Detroit, Mich.
- DALTON, ALBERT J., B.S., M.A., Ph.D., Lecturer in Histology, McGill University, Montreal, Canada.

- DANFORTH, CHARLES HASKELL, A.M., Ph.D. (Ex. Com. '27-'31, Second Vice-Pres. '36-'38), Professor of Anatomy, Stanford University, 607 Cabrillo Ave., Stanford University, Calif.
- DARDINSKI, VINCENT J., A.B., M.A., M.D., Ph.D., Associate Professor of Pathology, Georgetown Medical School; Director of Laboratories, Georgetown University Hospital, Georgetown University, School of Medicine, Washington, D. C.
- DARON, GARMAN H., B.S., Ph.D., Associate Professor of Anatomy, Georgetown University, School of Medicine, Washington, D. C.
- DARRACH, WILLIAM, A.B., A.M., M.D., Sc.D., LL.D., Professor of Clinical Surgery and Dean Emeritus of Faculty of Medicine, College of Physicians and Surgeons, Columbia University, 180 Fort Washington Ave., New York City.
- DART, RAYMOND A., M.D., Ch.M., M.Sc., Professor of Anatomy and Dean of Faculty of Medicine, University of the Witwatersrand, Johannesburg, Transvaal, So. Africa.
- DAVENPORT, H. A., M.D., Associate Professor of Anatomy, Northwestern University Medical School, Chicago, Ill.
- DAVIS, CARL L., M.D., Professor of Anatomy, University of Maryland School of Medicine, Lombard and Greene Sts., Baltimore, Md.
- DAVIS, WARREN B., M.D., D.Sc., F.A.C.S., Clinical Professor of Oral Surgery, Jefferson Medical College; Maxillo-Facial Surgeon, Jefferson Hospital; Consulting Maxillo-Facial Surgeon, Kensington Hospital and Frankford Hospital; 135 S. 18th St., Philadelphia, Pa.
- DAWSON, ALDEN B., A.B., Ph.D., D.Sc., Professor of Zoology and Chairman of the Department of Biology, Harvard University, Biological Laboratories, Harvard University, Cambridge, Mass.
- DAWSON, HELEN L., B.S., M.S., Ph.D., Associate in Anatomy, The State University of Iowa, College of Medicine, Iowa City, Ia.
- DAY, CAMERON D., Ph.D., Professor of Zoology, Westminster College, Fulton, Mo.
- DECARLO, JOHN, M.D., Associate in Topographic and Applied Anatomy, Jefferson Medical College, 1629 S. Broad St., Philadelphia, Pa.
- DEES-MATTINGLY, MARIE, A.B., B.S., M.D., Associate Professor of Anatomy, Tulane University of Louisiana, New Orleans, La.
- DE GARIS, CHARLES F., A.B., M.D., A.M., Ph.D., Professor of Anatomy and Head of the Department, University of Oklahoma, School of Medicine, Oklahoma City, Okla.
- DEIGNAN, STELLA L., B.S., M.S., Ph.D., Consultant, Work Projects Administration, Apt. 3, 1945 Calvert St., N.W., Washington, D. C.
- DEMPSEY, EDWARD W., Ph.D., Instructor in Physiology, Harvard Medical School, Boston, Mass.
- DEMPSTER, WILFRID TAYLOR, Sc.D., Instructor in Anatomy, University of Michigan Medical School, Ann Arbor, Mich.
- DERBYSHIRE, ARTHUR J., Ph.D., Assistant Professor of Anatomy, Wayne University School of Medicine, 1512 St. Antoine St., Detroit, Mich.
- DETWILER, SAMUEL RANDALL, Ph.B., A.M., Ph.D., M.S. (Hon.), (Ex. Com. '30-'34), Professor of Anatomy, Department of Anatomy, College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.

- DOAN, CHARLES A., B.S., M.D., Professor and Chairman, Department of Medicine, and Director of Medical Research, *Ohio State University, Kinsman Hall, Columbus, O.*
- DODDS, GIDEON S., M.A., Ph.D., Professor of Histology and Embryology, *West Virginia University Medical School, Morgantown, W. Va.*
- DOMM, LINCOLN V., A.B., Ph.D., Research Associate in Zoology, Whitman Laboratory for Experimental Zoology, *The University of Chicago, Chicago, Ill.*
- DONALDSON, JOHN C., Ph.B., M.D., Professor of Anatomy, *School of Medicine, University of Pittsburgh, Pittsburgh, Pa.*
- DORRIS, FRANCES, Ph.D., Research Assistant in Embryology, *Yale University, Osborn Zoological Laboratory, New Haven, Conn.*
- DOW, ROBERT S., M.D., Ph.D., Assistant Professor of Anatomy, *University of Oregon Medical School, Portland, Ore.*
- DOWNNEY, HAL, A.M., Ph.D., Professor of Anatomy, *University of Minnesota Medical School, Minneapolis, Minn.*
- DUBOIS, FRANKLIN S., A.B., M.S., M.D., *Silver Hill, New Canaan, Conn.*
- DUESBERG, JULES, M.D., Professor of Anatomy, *University of Liege, 22 quai Mativa, Liege, Belgium.*
- DUNCAN, DONALD, A.B., A.M., Ph.D., Professor of Anatomy, *University of Texas, Galveston, Tex.*
- DUSHANE, GRAHAM P., Ph.D., Instructor in Zoology, *The University of Chicago, Chicago, Ill.*
- EARLE, WILTON R., Ph.D., Cytologist, *National Cancer Institute, Bethesda, Md.*
- EASTLICK, HERBERT L., A.B., M.S., Ph.D., Assistant Professor of Zoology, *Washington State College, Pullman, Wash.*
- EDWARDS, EDWARD A., M.D., Instructor in Anatomy, *Harvard Medical School, 23 Bay State Rd., Boston, Mass.*
- EDWARDS, J. GRAHAM, M.A., Ph.D., Assistant Professor of Anatomy, *University of Buffalo School of Medicine, 24 High St., Buffalo, N. Y.*
- EDWARDS, LINDEN F., B.A., M.S., Ph.D., Professor of Anatomy, *Ohio State University College of Medicine, Hamilton Hall, Columbus, O.*
- ELFTMAN, HERBERT, Ph.D., Instructor in Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*
- ELLIOTT, RUSH, A.B., A.M., Ph.D., Professor of Anatomy, *Department of Zoology, Ohio University, 3 Marietta Ave., Athens, O.*
- ELWYN, ADOLPH, A.M., Associate Professor of Neuroanatomy, *Columbia University, 630 West 168th St., New York City.*
- EMERSON, HENRY S., B.A., Ph.D., Instructor in Anatomy, *University of Michigan, Ann Arbor, Mich.*
- EMERY, FREDERICK E., Ph.D., Assistant Professor of Physiology, *University of Buffalo, Buffalo, N. Y.*
- EMMEL, VICTOR M., A.B., Sc.M., Ph.D., Instructor in Anatomy, *The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*
- ENDERS, ROBERT K., Ph.D., Associate Professor, *Edward Martin Biological Laboratory, Swarthmore College, Swarthmore, Pa.*
- ENGLE, EARL THERON, A.B., A.M., Ph.D., Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*

- ESAKI, SHIRO, M.D., Ph.D., Member of Anatomical Institute, *Keio University, Sasuke 666, Kamakura, Japan.*
- ESSENBERG, J. M., Ph.D., Associate Professor of Anatomy, *Loyola University School of Medicine, Chicago, Ill.*
- ESSICK, CHARLES RHEIN, B.A., M.D., *700 Centre Ave., Reading, Pa.*
- ETKIN, WILLIAM, Ph.D., Instructor in Comparative Anatomy, *College of the City of New York, New York City.*
- ETTINGER, GEORGE H., M.D., C.M., Professor of Physiology, *Queen's University Kingston, Ontario, Canada.*
- EVANS, HERBERT MCLEAN, B.S., M.D., M.D. honoris causa, Freiberg (Ex. Com. '19-'22; Pres. '30-'32), Hertzstein Professor of Biology, Professor of Anatomy and Director of the Institute of Experimental Biology, *University of California, Berkeley, Calif.*
- EVANS, THOMAS HORACE, M.D., Research Professor of Anatomy, New York Medical College and Flower-Fifth Ave. Hospital; *350 South Main St., Freeport, N. Y.*
- EVERETT, JOHN W., A.B., Ph.D., Assistant Professor of Anatomy, *Duke University School of Medicine, Durham, N. C.*
- FANKHAUSER, GERHARD, Ph.D., Associate Professor of Biology, *Princeton University, Princeton, N. J.*
- FARIS, HERVEY S., A.B., A.M., Ph.D., M.D., Practicing Physician and Surgeon, *115 South Stone Ave., Tucson, Ariz.*
- FARRIS, EDMOND J., Ph.D., Associate in Anatomy, Executive Director, *The Wistar Institute of Anatomy, Woodland Ave. and 36th St., Philadelphia, Pa.*
- FIGGE, F. H. J., A.B., Ph.D., Associate Professor of Anatomy, *Yale University, School of Medicine, New Haven, Conn.*
- FLEXNER, LOUIS BARKHOUSE, S.B., M.D., Research Associate, *Department of Embryology, Carnegie Institution, Wolfe and Madison Sts., Baltimore, Md.*
- FOGG, LLOYD C., B.S., Ph.D., Assistant Professor of Histology and Embryology, *Boston University Medical School, Boston, Mass.*
- FOLEY, JAMES OWEN, M.S., Ph.D., Associate Professor of Anatomy, *University of Alabama School of Medicine, University, Ala.*
- FORBES, THOMAS R., Ph.D., Instructor in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- FORMAN, JONATHAN, A.B., M.D., Lecturer on Diseases Due to Allergy in the Ohio State University, *394 East Town St., Columbus, O.*
- FOUST, H. L., D.V.M., Professor of Veterinary Anatomy, *Iowa State College, Ames, Ia.*
- FOX, CLEMENT A., B.S., M.A., Ph.D., Instructor in Anatomy, *Marquette University, School of Medicine, Milwaukee, Wis.*
- FRANCIS, C. C., M.D., Senior Instructor in Anatomy, *Western Reserve University School of Medicine, Cleveland, O.*
- FRASER, DORIS A., A.B., M.S., Ph.D., Instructor in Biology, *Russell Sage College, Troy, N. Y.*
- FRAZER, JOHN ERNEST, D.Sc., F.R.C.S., Professor of Anatomy, University of London, *St. Mary's Hospital Medical School, London, W., England.*
- FRENCH, H. E., M.S., M.D., Professor of Anatomy and Dean of the School of Medicine, *University of North Dakota, Grand Forks, N. Dak.*

- FREUDENBERGER, CLAY B., Ph.D., M.D., Professor of Anatomy and Head of the Department, *University of Utah School of Medicine, Salt Lake City, Utah.*
- GAGE, SIMON HENRY, B.S., (Charter member; Ex. Com. '06-'11), Professor of Histology and Embryology, Emeritus, *Stimson Hall, Cornell University, Ithaca, N. Y.*
- GAMBLE, DEAN L., B.S., Ph.D., President, Ward's Natural Science Establishment, *302 North Goodman St., Rochester, N. Y.*
- GARCIA, ARTURO, A.B., M.D., Professor of Anatomy and Head of Department, College of Medicine, University of the Philippines, *619 Colorado St., Manila, P. I.*
- GARDNER, WILLIAM U., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, Yale University School of Medicine, 333 Cedar St., New Haven, Conn.*
- GATZ, ARTHUR J., B.A., M.A., Ph.D., Instructor in Zoology, *Carleton College, Northfield, Minn.*
- GAUNT, ROBERT, Ph.D., Assistant Professor of Biology, *New York University, New York City.*
- GEIST, FREDERICK D., M.D., Associate Professor of Anatomy, *University of Wisconsin, Science Hall, Madison, Wis.*
- GEORGE, RUGGLES KERR, B.A., M.B., D.P.H., Assistant Professor of Anatomy, *University of Toronto, Toronto 5, Canada.*
- GEORGE, WESLEY CRITZ, A.B., A.M., Ph.D., Professor of Histology and Embryology, *University of North Carolina Medical School, Chapel Hill, N. C.*
- GERSE, ISIDORE, B.S., Ph.D., Instructor in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- GEY, GEORGE O., B.S., M.D., Instructor in Surgery, Johns Hopkins Hospital and University, *Johns Hopkins Medical School, Baltimore, Md.*
- GLOBUS, J. H., B.S., M.D., Associate Professor of Neuro-Anatomy, *New York University, 1133 Fifth Ave., New York City.*
- GODWIN, MELVIN C., A.B., Ph.D., Assistant Professor of Anatomy and Histology, *West Virginia University Medical School, Morgantown, W. Va.*
- GOLDNER, JACQUES WILHELM, M.D., Associate in Pathology, *Albany Medical College, Albany, N. Y.*
- GOLDSMITH, JOSEPH B., M.A., Ph.D., Associate Professor of Histology and Embryology, *Oklahoma University School of Medicine, Oklahoma City, Okla.*
- GOMEZ, E. T., Ph.D., Assistant Physiologist, *Division of Nutrition and Physiology, Bureau of Dairy Industry, U. S. Department of Agriculture, Beltsville Research Center, Beltsville, Md.*
- GOODRICH, HUBERT BAKER, B.S., M.A., Ph.D., Professor of Biology, *Wesleyan University, Middletown, Conn.*
- GORDON, ALBERT S., Ph.D., Instructor in Biology, *Washington Square College, New York University, New York City.*
- GOSS, CHARLES MAYO, A.B., M.D., Professor of Anatomy, *University of Alabama, School of Medicine, Tuscaloosa, Ala.*
- GOULD, HARLEY NATHAN, A.B., A.M., Ph.D., Professor of Biology, *Newcomb College, Tulane University, New Orleans, La.*
- GRAFFLIN, ALLAN L., A.B., M.D., Assistant Professor of Anatomy, *Harvard Medical School, Boston, Mass.*

- GRANT, J. C. BOILEAU, M.B., Ch.B., F.R.C.S. (Edin.), Professor of Anatomy, *University of Toronto, Toronto, Canada.*
- GRANT, MADELEINE P., S.B., M.A., Ph.D., Member Teaching Staff in Zoology, *Sarah Lawrence College, Bronxville, N. Y.*
- GRAVES, WILLIAM W., M.D., Professor and Director, Department of Neuropsychiatry, *St. Louis University School of Medicine, St. Louis, Mo.*
- GRAY, MARY E., B.A., Ph.D., Assistant, *Department of Anatomy, Vanderbilt University, Nashville, Tenn.*
- GRAY, PETER, Ph.D., D.I.C., Associate Professor of Biology, *University of Pittsburgh, Pittsburgh, Pa.*
- GREENE, CHARLES W., A.M., Ph.D. Professor Emeritus of Physiology and Pharmacology, University of Missouri; Lecturer in Physiology, Leland Stanford Jr. University, *Stanford University, Calif.*
- GREENE, EUNICE C., A.M., Research Investigator, *Syracuse University School of Medicine, Syracuse, N. Y.*
- GREENE, WALTER FARREER, A.B. Ph.D., Associate Professor of Anatomy, *College of Medicine, Syracuse University, Syracuse, N. Y.*
- GREENHILL, J. P., B.S., M.D., F.A.C.S., Professor and Vice Chairman, Department of Obstetrics and Gynecology, Loyola University Medical School; Professor of Gynecology, Cook County Graduate School of Medicine; Attending Gynecologist, Cook County Hospital, *Suite 2805-06 Pittsfield Building, 55 East Washington St., Chicago, Ill.*
- GREEP, ROY O., M.S., Ph.D., Research Associate, *Squibb Institute for Medical Research, New Brunswick, N. J.*
- GREGORY, WILLIAM KING, A.B., A.M., Ph.D., Sc.D., Professor of Vertebrate Palaeontology, Columbia; Curator Departments of Comparative and Human Anatomy and Ichthyology, Research Associate in Anthropology and Palaeontology, *American Museum of Natural History, New York City.*
- GREULICH, WILLIAM W., M.A., Ph.D., Professor of Physical Anthropology and Anatomy and Director of the Brush Foundation, *Western Reserve University School of Medicine, 2109 Adelbert Rd., Cleveland, O.*
- GRODINSKY, MANUEL, B.Sc., M.D., Associate Professor of Anatomy, University of Nebraska, College of Medicine, *631 N. 58th St., Omaha, Neb.*
- GUDERNATSCH, F., Ph.D., Visiting Professor, Graduate School, *New York University, Washington Square East, New York City.*
- GUILD, STACY R., A.M., Ph.D. (Ex. Com. '39-), Associate Professor of Otology and Director of Otological Research Laboratory, Johns Hopkins University, *Johns Hopkins Hospital, Baltimore, Md.*
- GURDJIAN, ELISHA STEPHENS, A.B., M.S., M.D., Ph.D., F.A.C.S., Neuro-Surgeon, Grace Providence and Receiving Hospitals; Instructor in Surgery, Wayne University, *340 David Whitney Bldg., Detroit, Mich.*
- GUTHRIE, MARY J., A.B., A.M., Ph.D., Professor of Zoology, *210 Lefevre Hall, University of Missouri, Columbia, Mo.*
- GUTTMACHER, ALAN F., A.B., M.D., Associate Professor of Obstetrics, Johns Hopkins University, *1039 N. Calvert St., Baltimore, Md.*
- GUYER, MICHAEL F., Ph.D., LL.D., Professor of Zoology, *University of Wisconsin, Madison, Wis.*

- HAIR, GENE W., Ph.D., Instructor in Anatomy, *University of Buffalo, School of Medicine, Buffalo, N. Y.*
- HALL, B. VINCENT, A.B., Ph.D., Assistant Professor of Zoology, *University of Illinois, Urbana, Ill.*
- HALL, BYRON E., Ph.D., M.D., *Mayo Clinic, Rochester, Minn.*
- HALL, EDMUND K., D.Sc., Ph.D., Assistant Professor of Anatomy, *University of Louisville Medical School, Louisville, Ky.*
- HALL, E. RAYMOND, Ph.D., Associate Professor of Vertebrate Zoology and Curator of Mammals, *Museum of Vertebrate Zoology, University of California, Berkeley, Calif.*
- HALPERT, BÉLA, M.D., Assistant Professor of Pathology, *Louisiana State University School of Medicine, New Orleans, La.*
- HAM, ARTHUR W., M.B., Associate Professor of Anatomy, *University of Toronto, Toronto, Canada.*
- HAMBLÉN, E. C., M.D., Associate Professor of Obstetrics and Gynecology, *Duke University School of Medicine, Durham, N. C.*
- HAMBURGER, VIKTOR, Ph.D., Associate Professor of Zoology, *Washington University, St. Louis, Mo.*
- HAMILTON, JAMES B., Ph.D., Assistant Professor of Anatomy, *Yale University School of Medicine, 333 Cedar St., New Haven, Conn.*
- HAMLETT, GEORGE W. D., A.B., A.M., Ph.D., Research Agent, *U. S. Biological Survey, Carnegie Laboratory of Embryology, Baltimore, Md.*
- HAMMOND, WARNER S., Ph.D., Instructor in Anatomy, *Cornell University Medical College, New York City.*
- HANAN, ERNEST B., A.B., A.M., M.D., *Bolivar, Mo.*
- HANSON, FRANK BLAIR, A.B., A.M., Ph.D., Associate Director, *The Rockefeller Foundation, 49 West 49th St., New York City.*
- HARDESTY, IRVING, A.B., Ph.D., Sc.D. (Ex. Com. '10 and '12-'15, '22-'23), Emeritus Professor of Anatomy and Head of Department of Anatomy, *School of Medicine, Tulane University, 1301 Pine St., New Orleans, La.*
- HARE, WILLIAM K., A.B., M.S., Ph.D., Assistant Professor of Anatomy, *Cornell Medical College, New York City.*
- HARGITT GEORGE T., A.B., A.M., Ph.D., Sc.D., Professor of Zoology, *Biology Bldg., Duke University, Durham, N. C.*
- HARRIS, HENRY A., M.A., M.D., B.S., M.R.C.P., D.Sc., Professor of Anatomy, *The Anatomy School, The University, Cambridge, England.*
- HARRISON, FRANK, B.S., Ph.D., Instructor in Anatomy, *University of Tennessee, Memphis, Tenn.*
- HARRISON, ROSS GRANVILLE, Ph.D., M.D., Sc.D., (Pres. '12-'14), Chairman, National Research Council; Sterling Professor of Biology and Director of Osborn Zoological Laboratory Emeritus, *Yale University, New Haven, Conn.*
- HARTMAN, CARL G., Ph.D. (Ex. Com. '29-'33) (First Vice-Pres. '40-), Research Associate, *Carnegie Laboratory of Embryology, Johns Hopkins Medical School, Baltimore, Md.*
- HARVEY, BASIL COLEMAN HYATT, A.B., M.D., Professor of Anatomy and Dean of Students, Division of Biological Sciences, *The University of Chicago, Chicago, Ill.*

- HARWELL, CHARLES W., A.B., M.D., Assistant Professor in Micro-Anatomy, *Emory University, Emory, Ga.*
- HATERIUS, HANS OLIVER, A.B., M.S., Ph.D., Associate Professor of Physiology, *Wayne University College of Medicine, Detroit, Mich.*
- HAUSMAN, LOUIS, A.B., M.D., Associate Professor of Neurology, Cornell Medical College; Physician to Out-patients, New York Hospital; Associate Visiting Physician, Bellevue Hospital, *140 E. 54th St., New York City.*
- HEINBECKER, PETER, M.D., Associate Professor of Surgery, *Washington University Medical School, St. Louis, Mo.*
- HELDT, THOMAS J., A.B., A.M., M.D., Physician in Charge of Division of Neuropsychiatry, *Henry Ford Hospital, Detroit, Mich.*
- HELFF, O. M., B.S., M.S., Ph.D., Associate Professor of Biology, *New York University, New York City.*
- HELLBAUM, ARTHUR A., M.A., Ph.D., Associate Professor of Physiology, *University of Oklahoma, School of Medicine, Oklahoma City, Okla.*
- HERRICK, CHARLES JUDSON, Ph.D., (Ex. Com. '13-'17), Emeritus Professor of Neurology, The University of Chicago, *236 Morningside Drive, Grand Rapids, Mich.*
- HERTIG, ARTHUR T., M.D., Associate in Pathology and Instructor in Obstetrics, Harvard Medical School; Pathologist and Visiting Obstetrician to Out-patients, Boston Lying-in Hospital; Pathologist, Free Hospital for Women, Brookline, Mass.; *221 Longwood Ave., Boston, Mass.*
- HERTZLER, ARTHUR E., B.S., A.M., Ph.D., M.D., LL.D., D.Sc., F.A.C.S., Professor of Surgery, *University of Kansas, Halstead, Kan.*
- HETHERINGTON, ALBERT W., B.S., Ph.D., Instructor in Neurology, *Northwestern University Medical School, Chicago, Ill.*
- HETHERINGTON, DUNCAN CHARACTERIS, A.B., M.A., Ph.D., M.D., Associate Professor of Anatomy, *Duke University Medical School, Durham, N.C.*
- HEUSER, CHESTER H., A.M., Ph.D., Research Associate, Department of Embryology, Carnegie Institution, *Johns Hopkins Medical School, Baltimore, Md.*
- HIGGINS, GEORGE M., A.M., Ph.D., Associate Professor of Experimental Biology, *University of Minnesota, Mayo Foundation, Rochester, Minn.*
- HILL, JAMES PETER, D.Sc., F.R.S., Professor Emeritus of Embryology, *Royal College of Surgeons, Lincoln's Inn Fields, London, W.C. 2, England.*
- HILL, JOHN E., Ph.D., Assistant Curator of Mammals, *American Museum of Natural History, New York City.*
- HILL, ROBERT T., Ph.D., Assistant Professor of Anatomy, *Indiana University, Medical School, Bloomington, Ind.*
- HILTON, WILLIAM A., B.S., Ph.D., Professor of Zoology, Pomona College, Director *Laguna Marine Laboratory, Claremont, Calif.*
- HINES, MARION, A.B., Ph.D., Associate Professor of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- HINSEY, JOSEPH C., M.S., Ph.D. (Ex. Com. '40-), Professor of Anatomy, *Cornell University Medical College, 1300 York Ave., New York City.*
- HIRSCH, G. CHRISTIAN, Ph.D., Lector, Head of Department of Experimental Histology, *Zoological Laboratory. The University, Utrecht, Janskerkhof 3, Holland.*

- HISAW, FREDERICK L., A.B., A.M., Ph.D., LL.D., Professor of Zoology, Chairman of the Department of Biology, *Biological Laboratories, Harvard University, Cambridge, Mass.*
- HOADLEY, LEIGH, A.B., Ph.D., Professor of Zoology, *Harvard University, Cambridge, Mass.*
- HOERR, NORMAND L., A.B., Ph.D., M.D., Professor of Anatomy, *School of Medicine, Western Reserve University, 2109 Adelbert Rd., Cleveland, O.*
- HOGG, IRA D., B.A., M.A., Ph.D., Assistant Professor of Anatomy, Department of Anatomy, University of Pittsburgh School of Medicine, *617 Hampton Ave., Wilkensburg, Pa.*
- HOGUE, MARY J., Ph.D., Associate in Anatomy, *University of Pennsylvania Medical School, Philadelphia, Pa.*
- HOLLINSHEAD, W. HENRY, A.B., M.S., Ph.D., Assistant Professor of Anatomy, *Duke University School of Medicine, Durham, N. C.*
- HOLT, CAROLINE M., A.B., A.M., Ph.D., Professor of Biology and Public Health, *Simmons College, 38 Ridge Ave., Newton Centre, Mass.*
- HOLYOKE, EDWARD A., M.D., Ph.D., Assistant Professor of Anatomy, *University of Nebraska, College of Medicine, 42nd and Dewey Ave., Omaha, Neb.*
- HOOKER, CHARLES W., Ph.D., Instructor in Anatomy, *Yale University School of Medicine, New Haven, Conn.*
- HOOKER, DAVENPORT, Ph.D., Sc.D. (Ex. Com., '22-25) (Second Vice-Pres. '40-), Professor of Anatomy and Head of the Department, *School of Medicine, University of Pittsburgh, Pittsburgh, Pa.*
- HOPKINS, GRANT SHERMAN, Sc.D., D.V.M., Emeritus Professor of Comparative Veterinary Anatomy, *Cornell University, Ithaca, N. Y.*
- HOSKINS, MARGARET MORRIS, Ph.D., Associate Professor of Microscopic Anatomy, *New York University Dental College, 477 First Ave., New York City.*
- HOWE, HOWARD A., M.D., Instructor in Anatomy, *Johns Hopkins University School of Medicine, Baltimore, Md.*
- HOWELL, A. BRAZIER, Associate Professor of Anatomy, *Department of Anatomy, Johns Hopkins Medical School, Baltimore, Md.*
- HOWLAND, RUTH B., Ph.D., Associate Professor of Biology, *Washington Square College, New York University, Washington Square East, New York City.*
- HOY, WM. E., A.B., Ph.D., Professor of Biology and Head of the Department of Biology, *University of South Carolina, Columbia, S. C.*
- HEDLIČKA, ALEŠ, M.D., Sc.D., Curator, Division of Physical Anthropology, *United States National Museum, Smithsonian Institution, Washington, D. C.*
- HUBER, JOHN F., M.D., Ph.D., Associate Professor of Anatomy, *Temple University, Philadelphia, Pa.*
- HUGHSON, WALTER, S.B., M.D., Director Otological Research Laboratory, *Abington Memorial Hospital, Abington, Pa.*
- HUMMEL, KATHARINE P., Ph.D., *Department of Zoology, New Jersey College, New Brunswick, N. J.*
- HUMPHREY, RUFUS R., A.M., Ph.D., Professor of Anatomy, *School of Medicine, University of Buffalo, 24 High St., Buffalo, N. Y.*
- HUMPHREY, TRYPHENA, M.D., Ph.D., Instructor in Anatomy, *University of Pittsburgh Medical School, Pittsburgh, Pa.*

- HUNT, THOMAS E., Ph.D., Associate Professor of Histology and Embryology, *University of Alabama Medical School, University, Ala.*
- HUNTER OSCAR B., A.B., A.M., M.D., Professional Lecturer, Georgetown University, *1335 Eye St., N. W., Washington, D. C.*
- HUTCHINSON, CRANFORD, Ph.D., Associate, The Wistar Institute, *Morris Biological Farm, Bristol, Pa.*
- INGALLS, N. WILLIAM, B.S., M.D., Associate Professor of Anatomy, School of Medicine, *Western Reserve University, 3526 Edison Road, Cleveland Heights, O.*
- INGERSOLL, EVERETT H., B.S., M.S., Ph.D., Associate Professor of Anatomy, *Medical College of Virginia, Richmond, Va.*
- INGRAM, W. R., M.S., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, State University of Iowa, Iowa City, Ia.*
- JACKSON, CLARENCE M., M.S., M.D., LL.D., (Ex. Com. '10-'14, '23-'28, Vice-Pres. '16-'17. Pres. '22-'23), Professor and Head of the Department of Anatomy, *Institute of Anatomy, University of Minnesota, Minneapolis, Minn.*
- JACKSON, JOSEPH L., M.A., M.B., Professor of Anatomy, *University of Saskatchewan, Saskatoon, Canada.*
- JANES, RALPH G., Ph.D., Instructor in Anatomy, *Wayne University School of Medicine, 1512 St. Antoine St., Detroit, Mich.*
- JEFFERS, KATHARINE R., Ph.D., Assistant Professor of Zoology, *Duke University, Durham, N. C.*
- JENKINS, GEORGE B., M.D., Professor of Anatomy and Director of Anatomy Department, *George Washington University Medical School, Washington, D. C.*
- JOB, THESLE T., A.B., M.S., Ph.D., Professor of Anatomy, *Loyola University School of Medicine, 706 South Wolcott Ave., Chicago, Ill.*
- JOHNSON, FRANKLIN P., A.M., Ph.D., M.D., Associate Professor of Urology, *University of Oregon Medical School, 310 Medical Arts Building, Portland, Ore.*
- JOHNSON, MYRA L., A.B., A.M., Ph.D., Assistant Professor of Zoology, *Smith College, 41 West St., Northampton, Mass.*
- JOHNSON, SYDNEY E., Ph.D., M.D., F.A.C.P., Professor of Gross Anatomy, *School of Medicine, University of Louisville, 101 West Chestnut St., Louisville, Ky.*
- JOHNSTON, THOMAS BAILLIE, M.D., Professor of Anatomy, *University of London, Guy's Hospital Medical School, London, S. E. 1, England.*
- JOHNSTONE, PAUL N., A.B., M.D., Instructor in Surgery, *University of Kansas Medical School, 210 Argyle Bldg., Kansas City, Mo.*
- JONES, DAVID S., Ph.D., Associate in Anatomy, *Loyola University Medical School, 706 S. Wolcott, St., Chicago, Ill.*
- JONES, OLIVER P., Ph.D., Assistant Professor of Anatomy, *University of Buffalo, School of Medicine, 24 High St., Buffalo, N. Y.*
- JONES, RUSSELL, Ph.D., Assistant Professor of Anatomy, *Indiana University School of Medicine, Bloomington, Ind.*
- JORDAN, HARVEY ERNEST, A.M., Ph.D. (Ex. Com. '18-'21) (First Vice-Pres. '36-'38), Dean of the Department of Medicine, Professor of Anatomy and Director of the Anatomical Laboratories, *Pavilion VIII, East Lawn, University of Virginia, Charlottesville, Va.*

- JOY, EDWARD A., A.B., A.M., Ph.D., Associate Professor of Anatomy, *Tufts College Medical School, 416 Huntington Ave., Boston, Mass.*
- KAAN, HELEN WARTON, Ph.D., Associate Professor of Zoology, *Wellesley College, Wellesley, Mass.*
- KABAT, HERMAN, Ph.D., Instructor in Physiology, *University of Minnesota, Minneapolis, Minn.*
- KAMPMEIER, OTTO FREDERIC, A.B., Ph.D., M.D., Professor of Anatomy and Head of Department, *College of Medicine, University of Illinois, 1853 W. Polk St., Chicago, Ill.*
- KAPPERS, CORNELIUS UBBO ARIËNS, M.D., Sc.D., T.D., Director of the Central T.L.L. Institute for Brain Research of Holland; *Professor of Comparative Neurology, University of Amsterdam, Mauritskade 61, Amsterdam, Holland.*
- KEEGAN, J. JAY, A.M., M.D., Professor of Surgery, *University of Nebraska College of Medicine, 1234 Medical Arts Building, Omaha, Neb.*
- KEITH, SIR ARTHUR, M.D., LL.D., F.R.C.S., D.Sc., F.R.S., Master of *Buckston Browne Research Farm, Downe, Kent, England.*
- KELLOGG, REMINGTON, Ph.D., Curator, Division of Mammals, *U. S. National Museum, Washington, D. C.*
- KELLY, G. LOMBARD, A.B., B.S., M.D., Dean and Professor of Anatomy, *University of Georgia, School of Medicine, Augusta, Ga.*
- KERNAN, JOHN D., JR., A.B., M.D., Professor of Oto-Laryngology, *Columbia College of Physicians and Surgeons, 103 East 78th St., New York City.*
- KEY, J. ALBERT, B.S., M.D., Clinical Professor of Orthopedic Surgery, *Washington University, St. Louis, Mo.*
- KILLE, FRANK R., Ph.D., Assistant Professor of Zoology, *Swarthmore College, Swarthmore, Pa.*
- KIMMEL, DONALD L., Ph.D., Instructor in Anatomy, *School of Medicine, Louisiana State University, New Orleans, La.*
- KINDRED, JAMES ERNEST, A.B., A.M., Ph.D., Associate Professor of Anatomy, *University of Virginia Medical School, Box 1341, University Station, Charlottesville, Va.*
- KING, HELEN DEAN, A.M., Ph.D., Member of The Wistar Institute (Experimental Zoology), *The Wistar Institute of Anatomy, 36th St. and Woodland Ave., Philadelphia, Pa.*
- KING, JESSIE L., Ph.D., Professor of Physiology, *Goucher College, Baltimore, Md.*
- KINGERY, HUGH McMILLAN, A.B., A.M., Ph.D., Associate Professor of Anatomy, *School of Medicine, University of Colorado, Denver, Colo.*
- KINGSBURY, BENJAMIN F., A.B., Ph.D., M.D., D.Sc. (Ex. Com. '22-'25, Vice-Pres. '32-'34), Professor of Histology and Embryology, *Cornell University, 2 South Ave., Ithaca, N. Y.*
- KINGSLEY, D. M., M.S., Ph.D., M.D., Assistant Visiting Surgeon to Charity Hospital, *15 Fontainebleau Drive, New Orleans, La.*
- KIRKHAM, WILLIAM BARRI, Ph.D., Consulting Director, *Museum of Natural History 220 State St., Springfield, Mass.*
- KIRKMAN, HADLEY, A.B., S.M., Ph.D., Assistant Professor of Anatomy, *Stanford University, Calif.*
- KIRSCHBAUM, ARTHUR, Ph.D., Instructor in Anatomy, *Yale University School of Medicine, 333 Cedar St., New Haven, Conn.*

- KNISELY, MELVIN H., Ph.D., Assistant Professor of Anatomy, *The University of Chicago, Chicago, Ill.*
- KNOUFF, R. A., B.A., M.A., Ph.D., Professor of Anatomy, *Department of Anatomy, Ohio State University, Columbus, O.*
- KOCH, JOHN C., B.S., M.D., *265 Euclid Ave., East, Detroit, Mich.*
- KOFOID, CHARLES ATWOOD, A.B., Ph.D., Sc.D., LL.D., Professor of Zoology Emeritus, *Room 5090, Life Sciences Bldg., University of California, Berkeley, Calif.*
- KORNHAUSER, S. I., M.A., Ph.D., Professor of Anatomy and Executive Secretary, *University of Louisville, School of Medicine, 101 W. Chestnut St., Louisville, Ky.*
- KRAFKA, JOSEPH, JR., Ph.D., M.D., Professor of Microscopic Anatomy, *University of Georgia School of Medicine, Augusta, Ga.*
- KRAUSE, ALLEN KRAMER, A.B., A.M., M.D., Litt.D., Editor, *American Review of Tuberculosis, 96 Medway St., Providence, R. I.*
- KREHBIEL, ROBERT H., M.S., Ph.D., Instructor in Anatomy, *University of Illinois, College of Medicine, 1853 W. Polk St., Chicago, Ill.*
- KRIEG, WENDELL J. S., Ph.D., Assistant Professor of Anatomy, *New York University, College of Medicine, New York City.*
- KROGMAN, WILTON M., Ph.D., Associate Professor of Anatomy and Physical Anthropology, *Department of Anatomy, The University of Chicago, Chicago, Ill.*
- KROPP, BENJAMIN, Ph.D., Lecturer in Embryology, *Queen's University Medical School, Kingston, Ontario, Canada.*
- KUHLENBECK, HARTWIG, M.D., Ph.D., Professor of Anatomy, *Woman's Medical College of Pennsylvania, Philadelphia, Pa.*
- KUNKEL, BEVERLY WAUGH, Ph.B., Ph.D., Professor of Biology, *Lafayette College, Easton, Pa.*
- KUNTZ, ALBERT, Ph.D., M.D. (Second Vice-Pres. '38-'39; First Vice-Pres. '39-'40), Professor of Micro-anatomy, *St. Louis University Medical School, 1402 South Grand Ave., St. Louis, Mo.*
- KUTCHIN, MRS. HARRIET LEHMANN, A.M., "*The Maplewood*," *Green Lake, Wis.*
- LACHMAN, ERNEST, M.D., Associate Professor of Anatomy, *University of Oklahoma School of Medicine, Oklahoma City, Okla.*
- LAMBERT, AVERY ELDORUS, B.S., Ph.D., Professor of Histology, *State University of Iowa Medical School, Room 360, Medical Laboratories Bldg., Iowa City, Ia.*
- LANGWORTHY, ORTHELLO R., A.B., A.M., M.D., Associate Professor of Neurology, *Johns Hopkins University, Johns Hopkins Hospital, Baltimore, Md.*
- LANIER, RAYMOND R., B.A., Ph.D., Assistant in Anatomy, *Washington University, St. Louis, Mo.*
- LARSELL, OLOF, A.M., Ph.D., (Ex. Com. '38-), Professor of Anatomy, Dean of Graduate Division, *Oregon State System of Higher Education, University of Oregon Medical School, Portland, Ore.*
- LASSEK, ARTHUR M., M.S., Ph.D., M.D., Professor of Anatomy, *Medical College of the State of South Carolina, Charleston, S. C.*
- LATIMER, HOMER B., A.M., Ph.D., Professor of Anatomy, *Medical School, University of Kansas, Lawrence, Kan.*

- LATTA, JOHN S., A.B., Ph.D., Professor of Anatomy, University of Nebraska, College of Medicine, *Forty-second St. and Dewey Ave., Omaha, Neb.*
- LAWLESS, JOHN J., M.A., Ph.D., M.D., Assistant Professor of Anatomy, *Medical Branch, University of Texas, Galveston, Tex.*
- LEATHAM, JAMES H., B.S., M.A., Ph.D., Research Associate in Anatomy, *Columbia University, College of Physicians and Surgeons, New York City.*
- LEBLOND, C. P., M.D., *University of Rochester School of Medicine and Dentistry, Rochester, N. Y.*
- LEE, FERDINAND CHRISTIAN, A.B., M.D., Associate Professor of Surgery, *Johns Hopkins Hospital, Baltimore, Md.*
- LEONARD, SAMUEL, Ph.D., Assistant Professor of Zoology, *Rutgers University, New Brunswick, N. J.*
- LEVENSTEIN, IRVING M., Ph.D., Assistant Instructor in Biology, *Washington Square College, New York University, New York City.*
- LEVI, GIUSEPPE, M.D., Former Professor of Anatomy, *Institut d'Anatomie pathol., 1, rue des Bonnes Villes, Liege, Belgique.*
- LEWIS, DEAN D., M.D., Sc.D., LL.D., Professor Emeritus of Surgery, *Johns Hopkins University, 210 Goodwood Gardens, Roland Park, Baltimore, Md.*
- LEWIS, FREDERIC T., A.M., M.D., (Ex. Com. '09-'13, Vice-Pres. '14-'16; Pres. '36-'38), Professor of Comparative Anatomy, *Harvard Medical School, Boston, Mass.*
- LEWIS, MARGARET REED, *The Wistar Institute, 36th St. and Woodland Ave., Philadelphia, Pa.*
- LEWIS, WARREN HARMON, B.S., M.D., (Ex. Com. '09-'11, '14-'17, Pres. '34-'36), *The Wistar Institute, 36th St. and Woodland Ave., Philadelphia, Pa.*
- LILLIE, FRANK RATTRAY, Ph.D., Sc.D., Andrew MacLeish Distinguished Service Professor of Embryology, Department of Zoology (Emeritus), *The University of Chicago; President of Marine Biological Laboratory, Woods Hole, Mass.; 5801 Kenwood Ave., Chicago, Ill.*
- LIMSON, MARCIANO, M.D., Associate Professor of Anatomy, *College of Medicine, University of the Philippines, Manila, P. I.*
- LINELL, ERIC AMBROSE, Ch.B., M.D., Professor of Neuropathology, *Banting Institute, University of Toronto, Canada.*
- LIPSHUTZ, BENJAMIN, M.D., Assistant Professor in Neuro-anatomy, *Jefferson Medical College, 1007 Spruce St., Philadelphia, Pa.*
- LITTLE, CLARENCE COOK, B.A., M.S., Sc.D., LL.D., Litt.D., Director, *Jackson Memorial Laboratory, Bar Harbor, Me.*
- LOOPER, JAMES BURDINE, Ph.D., M.D., Professor of Anatomy, *University of Mississippi, Oxford, Miss.*
- LOOSLI, CLAYTON G., Ph.D., M.D., Instructor in Medicine, *School of Medicine, The University of Chicago, 950 East 59th St., Chicago, Ill.*
- LORD, FREDERIC P., A.B., M.D., Professor of Anatomy, *Dartmouth Medical School, 39 College St., Hanover, N. H.*
- LORENTE DE NÓ, RAPHAEL, M.D., Associate Member, *Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City.*
- LOTH, EDWARD, M.D., Ph.D., Professor of Anatomy and Director of Anatomical Institute, *University of Warsaw, 5 Chalubinski Str., Warsaw, Poland.*

- LOW, FRANK NORMAN, A.B., Ph.D., Assistant Professor of Anatomy, *University of North Carolina Medical School, Chapel Hill, N. C.*
- LOWREY, LAWSON GENTRY, A.M., M.D., Psychiatrist, Brooklyn Hebrew Orphan Asylum and Travelers Aid Society, *25 West 54th St., New York City.*
- LUCAS, ALFRED M., Ph.D., Associate Professor of Zoology, *Iowa State College, Ames, Ia.*
- LUCAS, MIRIAM SCOTT, Ph.D., *Department of Zoology, Iowa State College, Ames, Ia.*
- LYONS, WILLIAM R., Ph.D., Assistant Professor of Anatomy, *University of California, Berkeley, Calif.*
- MACCALLUM, DANIEL BARTLETT S.B., Ph.D., M.D., Professor of Anatomy, *University of Southern California Medical School, Los Angeles, Calif.*
- MACCARDLE, R. C., B.S., Ph.D., Research Associate in Cytology and Dermatology, *Washington University School of Medicine, St. Louis, Mo.*
- MACEWEN, EWEN, B.S., M.S., M.D., Professor of Anatomy, Director of the Anatomical Laboratories, Dean, College of Medicine, *University of Iowa, Iowa City, Ia.*
- MACFALL, CLAUDE M., Ph.D., Professor of Anatomy, *George Washington Medical School, 1335 H Street, N. W., Washington, D. C.*
- MACFARLAND, FRANK MACE, A.M., Ph.D., Emeritus Professor of Histology, Leland Stanford Junior University; President California Academy of Sciences, San Francisco; *775 Santa Ynez St., Stanford University, Calif.*
- MCCLENDON, JESSE F., Ph.D., Research Professor of Physiology, *Hahnemann Medical College, 235 North 15th St., Philadelphia, Pa.*
- MCCLUNG, CLARENCE E., Ph.G., A.B., A.M., Ph.D., Emeritus Professor of Zoology, *University of Pennsylvania, Philadelphia, Pa.*
- MCCLURE, CHARLES FREEMAN WILLIAMS, A.M., Sc.D. (Vice-Pres. '10-'11, Ex-Com. '12-'16, Pres. '20-'21), Professor Emeritus of Comparative Anatomy, *Princeton University, Princeton, N. J.*
- MCCOTTER, ROLLO E., M.D., Professor of Anatomy, Medical School, University of Michigan, *Lakewood R. R. 1, Ann Arbor, Mich.*
- MCCRADY, EDWARD, JR., A.B., M.S., Ph.D., Professor of Biology, *University of the South, Sewanee, Tenn.*
- MCGILL, CAROLINE, A.B., A.M., Ph.D., M.D., Physician *58 W. Quartz, Butte, Mont.*
- MCKEEN, ALICE CURWEN, Ph.D., *258 Walnut St., Brookline, Mass.*
- MCKENZIE, FRED F., A.M., Ph.D., Assistant Professor of Animal Husbandry, *Schweitzer Hall, University of Missouri, Columbia, Mo.*
- MCKIBBEN, PAUL S., Ph.D., Sc.D., LL.D. (Ex. Com. '31-'35), Professor of Anatomy and Dean, School of Medicine, *University of Southern California, Los Angeles, Calif.*
- MCKINNEY, ROSCOE L., Ph.D., Professor of Anatomy, *Howard University, Washington, D. C.*
- MACKLIN, C. C., M.B., M.D., M.A., Ph.D., F.R.S.C. (Ex. Com. '28-'32), Professor of Histology and Embryology, *University of Western Ontario Medical School, London, Ontario, Canada.*
- MACLEAN, BERNICE L., A.B., Ph.D., Assistant Professor of Zoology, Department of Biological Sciences, *Hunter College, 2 Park Ave., New York City.*

- MAGATH, THOMAS BYRD, M.S., Ph.D., M.D., Consulting Physician, Clinical Laboratories, Mayo Clinic and Professor of Pathology and Parasitology, University of Minnesota, *Mayo Clinic, Rochester, Minn.*
- MAGOUN, H. W., Ph.D., Associate Professor of Neurology, *Northwestern University Medical School, Chicago, Ill.*
- MAGRUDER, SAMUEL R., B.S., M.A., Ph.D., Assistant Professor of Anatomy, *Tufts College Medical School, 416 Huntington Ave., Boston, Mass.*
- MAHON, ELEANOR CONWAY, Ph.D., *Iron River, Mich.*
- MAINLAND, DONALD, M.B., Ch.B., D.Sc., F.R.S.E., *Department of Anatomy, Dalhousie University, Halifax, Nova Scotia, Canada.*
- MALONE, EDWARD F., A.B., M.D. (Ex. Com. '31-'35), Francis Brunning Professor of Anatomy, *University of Cincinnati, College of Medicine, Eden Ave., Cincinnati, O.*
- MANTER, JOHN T., Ph.D., Assistant Professor of Anatomy, *University of South Dakota, School of Medicine, Vermillion, S. Dak.*
- MARK, EDWARD LAURENS, Ph.D., LL.D., Hersey Professor of Anatomy (Emeritus), Harvard University and Trustee of the Bermuda Biological Station for Research, Inc., *109 Irving St., Cambridge, Mass.*
- MARKEE, J. E., Ph.D., Associate Professor of Anatomy, *Stanford University, Stanford, Calif.*
- MARSHALL, CLYDE, M.D., Assistant Professor in Anatomy, Yale University School of Medicine, *333 Cedar St., New Haven, Conn.*
- MARTIN, CECIL P., M.A., M.B., D.Sc., Professor of Anatomy, *McGill University, Montreal, Canada.*
- MARTIN, JOHN, B.S., M.S., M.B., M.D., Associate in Surgery, *Northwestern University Medical School, Chicago, Ill.*
- MASON, KARL E., Ph.D., Professor of Anatomy, *The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*
- MASTIN, EDWARD V., M.S., M.D., Instructor in Surgery, *St. Louis University Medical School, St. Louis, Mo.*
- MATAS, RUDOLPH, M.D., LL.D., Sc.D., F.R.C.S. Eng. (hon.), (Charter member), Professor of Surgery (1894-1927), Emeritus (1927-), and Demonstrator of Anatomy (1885-1894), *Tulane University, 2255 St. Charles Ave., New Orleans, La.*
- MATTHEWS, SAMUEL A., M.A., Ph.D., Assistant Professor of Biology, *Williams College, Williamstown, Mass.*
- MAVOR, JAMES WATT, B.A., M.A., Ph.D., Professor of Biology, *Union College, Schenectady, N. Y.*
- MEAD, HAROLD TUPPER, B.A., M.S., Ph.D., Professor of Biology, *Limestone College, Gaffney, S. C.*
- MEADER, RALPH G., Ph.D., Assistant Professor of Anatomy, Yale University School of Medicine, *333 Cedar St., New Haven, Conn.*
- MEAKER, SAMUEL R., A.B., A.M., M.D., M.R.C.S. (Eng.), F.A.C.S., F.C.O.G., Professor of Gynaecology, *Boston University School of Medicine, 475 Commonwealth Ave., Boston, Mass.*
- MENDELSON, WILLIAM, M.S., M.D., *442 Temple St., New Haven, Conn.*
- MENKIN, VALY, M.D., Instructor in Pathology, *Harvard Medical School, Boston, Mass.*

- MERRIMAN, DANIEL, B.S., M.S., Ph.D., Instructor in Biology, *Osborn Zoological Laboratory, Yale University, New Haven, Conn.*
- METTLER, FRED A., A.B., A.M., M.D., Ph.D., Professor of Anatomy, University of Georgia Medical School (on leave of absence), *New York Neurologic Institute, New York City.*
- MEYER, ADOLF, M.D., LL.D., Professor of Psychiatry and Director of the Henry Phipps Psychiatric Clinic, *Johns Hopkins Hospital, Baltimore, Md.*
- MEYER, ARTHUR W., S.B., M.D. (Ex. Com. '12-'16, Vice-Pres. '24-'25), Professor of Anatomy Emeritus, Leland Stanford Junior University, *121 Waverly St., Palo Alto, Calif.*
- MEYER, ROLAND K., A.B., Ph.D., Professor of Zoology, *Department of Zoology, University of Wisconsin, Madison, Wis.*
- MICHELS, NICHOLAS A., M.A., D.Sc., Associate Professor of Anatomy, *The Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia, Pa.*
- MIDLO, CHARLES, M.D., Assistant Professor of Anatomy, *Louisiana State University, Medical Center, New Orleans, La.*
- MILLER, JAMES A., A.B., Ph.D., Instructor in Anatomy, *University of Michigan, Ann Arbor, Mich.*
- MILLER, ROLAND E., B.S., M.S., Ph.D., Assistant Professor of Anatomy, *School of Medical Sciences, Wake Forest College, Wake Forest, N. C.*
- MILLER, RUTH ALEXANDER, A.B., Ph.D., Instructor in Anatomy, College of Physicians and Surgeons, Columbia University, *630 W. 168th St., New York City.*
- MILLER, RUTH N., A.B., M.D., Associate Professor of Anatomy, *Woman's Medical College of Pennsylvania, Philadelphia, Pa.*
- MILLER, SHIRLEY P., A.B., A.M., Ph.D., Assistant Professor of Anatomy, *Anatomical Institute, University of Minnesota, Minneapolis, Minn.*
- MINCKLER, JEFF, A.B., M.A., Ph.D., Instructor in Anatomy, *Creighton University, Omaha, Neb.*
- MOODY, ROBERT ORTON, B.S., M.D., Emeritus Professor of Anatomy, University of California, *2826 Garber St., Berkeley, Calif.*
- MOORE, CARL R., A.M., Ph.D., Professor of Zoology, *Hull Zoological Laboratory, The University of Chicago, Chicago, Ill.*
- MORGAN, LAWRENCE ONIS, A.B., A.M., Ph.D., Associate Professor of Anatomy, *University of Cincinnati, College of Medicine, Eden Ave., Cincinnati, O.*
- MORRILL, CHARLES V., A.M., Ph.D., Associate Professor of Anatomy, *Cornell University Medical College, 1300 York Ave., New York City.*
- MORTENSEN, OTTO A., B.S., M.D., Associate Professor of Anatomy, *University of Wisconsin, Madison, Wis.*
- MORTON, DUDLEY JOY, M.D., Associate Professor, Department of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*
- MORTON, THOMAS H., Ph.D., Assistant Professor of Biology, *Niagara University, Niagara University, N. Y.*
- MOSSMAN, HARLAND W., Ph.D., Associate Professor of Anatomy, *Science Hall, University of Wisconsin, Madison, Wis.*

- MYERS, BURTON D., A.M., M.D., Professor of Anatomy (retired), Indiana University of Medicine, *424 N. Walnut St., Bloomington, Ind.*
- MYERS, J. ARTHUR, M.S., Ph.D., M.D., Professor of Preventive Medicine and Chief of Chest Clinic, *University of Minnesota Medical School, Minneapolis, Minn.*
- NACHTRIEB, HENRY FRANCIS, B.S., Professor Emeritus, Animal Biology, University of Minnesota, *2448 Cedar St., Berkeley, Calif.*
- NAHM, LAURA J., Ph.D., Professor of Biology, *Junior College of Flat River, Flat River, Mo.*
- NAÑAGAS, JUAN CANCIO, M.D., Professor of Anatomy, College of Medicine, University of the Philippines, *719 Florida St., Manila, P. I.*
- NELSON, ROSE C., B.A., M.A., Ph.D., Associate Fellow and Instructor in Pediatric Research, *Children's Hospital Research Foundation, Cincinnati, O.*
- NELSON, WARREN O., A.B., Sc.M., Ph.D., Professor and Head of the Department of Anatomy; Chairman of the Division of Basic Medical Sciences, *Wayne University, School of Medicine, 1512 St. Antoine St., Detroit, Mich.*
- NICHOLAS, JOHN SPANGLER, M.S., Ph.D., Sterling Professor of Biology, *Osborn Zoological Laboratory, Yale University, New Haven, Conn.*
- NOBACK, GUSTAVE J., B.S., M.A., Ph.D., Professor of Anatomy, New York University, *477 First Ave., New York City.*
- NONDEZ, JOSÉ F., M.S., Sc.D., Professor of Anatomy, Cornell University Medical College, *1300 York Ave., New York City.*
- NORRIS, H. W., A.B., Sc.D., Research Professor of Zoology, *Grinnell College, Grinnell, Ia.*
- OBENCHAIN, JEANETTE B., Ph.D., Research Associate, Department of Anatomy, *The University of Chicago, Chicago, Ill.*
- ODIORNE, JOSEPH M., B.S., M.A., Ph.D., Instructor in Anatomy, *Georgetown University School of Medicine, Washington, D. C.*
- O'DONOGHUE, CHARLES H., D.Sc., F.Z.S., F.R.S.E., Professor of Zoology, *University of Reading, Reading, England.*
- O'LEARY, JAMES L., Ph.D., M.D., Associate Professor of Cytology, *Washington University School of Medicine, St. Louis, Mo.*
- OPPENHEIMER, JANE M., Ph.D., Instructor in Biology, Bryn Mawr College, *235 South 15th St., Philadelphia, Pa.*
- OSBORN, CLINTON M., A.B., A.M., Ph.D., Instructor in Histology and Embryology, *Department of Anatomy, Ohio State University, Columbus, O.*
- OSTERUD, HJALMAR L., A.B., A.M., Ph.D., Professor of Anatomy, *Medical College of Virginia, Richmond, Va.*
- OVERHOLSER, MILTON D., A.B., A.M., Ph.D., M.D., Professor of Anatomy and Chairman of Department, *University of Missouri, Columbia, Mo.*
- PAFF, GEORGE H., Ph.D., Assistant Professor of Anatomy, *Long Island College of Medicine, 350 Henry St., Brooklyn, N. Y.*
- PALMER, CARROLL E., M.A., Ph.D., M.D., Medical Officer in Charge, Office of Child Hygiene, *U. S. Public Health Service, Bethesda, Md.*
- PALMER, DWIGHT M., B.Sc., M.Sc., M.D., Assistant Professor of Anatomy and Neuro-psychiatry, *Ohio State University, Columbus, O.*
- PANKRATZ, DAVID S., A.B., A.M., Ph.D., M.D., Professor of Anatomy, *University of Mississippi, Box 15, University, Miss.*

- PAPANICOLAOU, GEORGE N., Ph.D., M.D., Associate Professor of Anatomy, *Cornell University Medical College, 1300 York Ave., New York City.*
- PAPEZ, JAMES WENCESLAS, B.A., M.D., Professor of Anatomy, *Cornell University, Ithaca, N. Y.*
- PARKER, ALICE E., Ph.D., Instructor in Anatomy, *University of Colorado School of Medicine, Denver, Colo.*
- PARKER, GEORGE HOWARD, D.Sc., Professor of Zoology, Emeritus, *Harvard University, 16 Berkeley St., Cambridge, Mass.*
- PARKER, RAYMOND C., B.A., Ph.D., Head of Virus Division, *Biological Laboratories, E. E. Squibb & Sons, New Brunswick, N. J.*
- PARKES, A. S., D.Sc., F.R.S., Member, *National Institute for Medical Research, Hampstead, London, N.W. 3, England.*
- PATEK, PAUL R., B.A., Ph.D., Instructor in Anatomy, *School of Medicine, University of Southern California, Los Angeles, Calif.*
- PATTEN, BRADLEY MERRILL, A.M., Ph.D. (Second Vice-Pres. '34-'36), Professor of Anatomy, *University of Michigan, Ann Arbor, Mich.*
- PATTERSON, JOHN THOMAS, B.S., Ph.D., Professor of Zoology and Director of Research in Zoology, *University of Texas, 1908 Cliff St., Austin, Tex.*
- PEARSON, ANTHONY A., M.A., Ph.D., Assistant Professor of Anatomy, *Loyola University School of Medicine, 706 South Wolcott Ave., Chicago, Ill.*
- PENCHARZ, RICHARD I., Ph.D., Instructor, Department of Obstetrics and Gynecology, *University of California Medical School, Ranchero Rd., Kent Woodlands, Kentfield, Calif.*
- PERKINS, ORMAN C., A.B., A.M., M.D., F.A.C.P., Professor of Neurology, *Long Island College of Medicine, 829 Carroll St., Brooklyn, N. Y.*
- PFEIFFER, CARROLL A., Ph.D., Assistant Professor of Anatomy, *Yale University School of Medicine, 333 Cedar St., New Haven, Conn.*
- PHELPS, DORIS H., A.B., M.A., Ph.D., Research Associate in Gynecology and Obstetrics, *Vanderbilt University School of Medicine, Nashville, Tenn.*
- PIATT, JEAN, B.S., M.A., Ph.D., Instructor, Department of Anatomy, *Medical College, University of Vermont (on leave). Biological Division, Edmund Niles Huyck Preserve, Rensselaerville, Albany County, N. Y.*
- PICK, JOSEPH, M.D., Instructor in Anatomy, *New York University, College of Medicine, New York City.*
- PIERSOL, WILLIAM HUNTER, B.A., M.B., Professor of Histology and Embryology, *University of Toronto, 35 Dunvegan Road, Toronto, Canada.*
- PINCUS, GREGORY, Sc.D., Visiting Professor of Experimental Zoology, *Clark University, Worcester, Mass.*
- PLAGGE, JAMES C., S.B., Ph.D., Instructor in Gross Anatomy, *University of Maryland School of Medicine, Baltimore, Md.*
- PLISKE, EDWARD C., Ph.D., Instructor in Anatomy, *Medical School, University of North Carolina, Chapel Hill, N. C.*
- POHLMAN, AUGUSTUS G., M.D. (Ex. Com. '32-'36), Associate Clinical Professor of Otolaryngology, *University of Southern California, 2202 W. Third St., Los Angeles, Calif.*
- POLYAK, STEPHEN, M.D., Associate Professor of Anatomy, Department of Anatomy, *The University of Chicago, Chicago, Ill.*

- POYNTER, CHARLES W. M., B.S., M.D., (Ex. Com. '19-'22), Professor of Anatomy, and Dean of the College of Medicine, University of Nebraska, *Forty-second and Dewey Ave., Omaha, Neb.*
- PRATT, JEAN PAUL, M.D., Surgeon in Charge of Divisions of Gynecology and Obstetrics, *Henry Ford Hospital, Detroit, Mich.*
- PRYOR, JOSEPH WILLIAM, M.D., Emeritus Professor of Anatomy and Physiology, University of Kentucky, *417 West Second St., Lexington, Ky.*
- PUCKETT, WILLIAM O., M.A., Ph.D., Assistant Professor of Biology, *Princeton University, Princeton, N. J.*
- QUIRING, D. P., M.A., Ph.D., Assistant Professor of Biology, *Western Reserve University, Cleveland, O.*
- RADASCH, HENRY E., M.S., M.D., Professor of Histology and Embryology, Jefferson Medical College, *Daniel Baugh Institute of Anatomy, Eleventh and Clinton Sts., Philadelphia, Pa.*
- RAMSAY, ANDREW J., Ph.D., Assistant Professor of Histology and Embryology, *Jefferson Medical College, Philadelphia, Pa.*
- RANSON, STEPHEN W., M.D., Ph.D. (Ex. Com. '21-'24, Vice-Pres. '26-'28, Pres. '38-'40), Professor and Director of Neurological Institute, Northwestern University Medical School, *303 East Chicago Ave., Chicago, Ill.*
- RASMUSSEN, ANDREW T., Ph.D., (Ex. Com. '34-'38), Professor of Neurology, *Institute of Anatomy, University of Minnesota, Minneapolis, Minn.*
- RAVEN, HENRY C., Associate Curator, Department of Comparative and Human Anatomy, *American Museum of Natural History, 77th St. and Central Park West, New York City.*
- RAWLES, MARY E., A.B., S.M., Ph.D., Research Associate (Embryology), *Department of Biology, Johns Hopkins University, Baltimore, Md.*
- RAWLINSON, HERBERT E., M.D., M.Sc., Ph.D., Associate Professor of Anatomy, *University of Alberta, Edmonton, Alberta, Canada.*
- REAGAN, FRANKLIN P., Ph.D., Lecturer in Anatomy, Hospitals Centre, Medical School, *The University, Edmund St., Birmingham, England.*
- REECE, RALPH PARLETTE, B.Sc., M.Sc., Assistant Professor of Dairy Husbandry, *New Jersey Agricultural Experiment Station, New Brunswick, N. J.*
- REESE, JOHN D., M.D., Assistant Professor of Anatomy, *University of California, Berkeley, Calif.*
- DE RÉNYI, GEORGE S., M.D., Associate Professor of Anatomy, University of Pennsylvania, School of Medicine, *212 S. 39th St., Philadelphia, Pa.*
- REVELL, DANIEL GRAISBERRY, A.B., M.B., Professor Emeritus of Anatomy, University of Alberta, *9105 112 St., South Edmonton, Alberta, Canada.*
- REYNOLDS, SAMUEL R. M., M.A., Ph.D., Associate Professor of Physiology, *Long Island College of Medicine, 350 Henry St., Brooklyn, N. Y.*
- RHINEHART, D. A., A.M., M.D., Professor of Applied Anatomy, University of Arkansas, *4 Armistead Rd., Little Rock, Ark.*
- RICE, EDWARD LORANUS, A.B., Ph.D., Sc.D., Professor of Zoölogy, *Ohio Wesleyan University, Delaware, O.*
- RICHARDSON, DOROTHY, A.B., Ph.D., Associate Professor of Zoology, *Rockford College, Rockford, Ill.*
- RIENHOFF, WILLIAM F., JR., A.B., M.D., Associate Professor of Surgery, Johns Hopkins Hospital, *1201 North Calvert St., Baltimore, Md.*

- RINGOEN, ADOLPH R., A.M., Ph.D., Associate Professor of Zoölogy, *Department of Zoölogy, University of Minnesota, Minneapolis, Minn.*
- RIOCH, DAVID McK., A.B., M.D., Professor of Neurology, *Washington University School of Medicine, St. Louis, Mo.*
- DE ROBERTIS, EDUARDO D. P., Member of Staff of Institute of General Anatomy, University of Buenos Aires, Argentina; Rockefeller Fellow, *Department of Anatomy, Johns Hopkins Medical School, Baltimore, Md.*
- ROBERTS, JOSEPH T., B.A., M.S., M.D., Instructor in Anatomy, Instructor in Medicine, Assistant in Pediatrics, *University of Texas, Medical Branch, Galveston, Tex.*
- ROBINSON, BYRON L., M.A., M.D., Professor of Anatomy, *University of Arkansas Medical School, Little Rock, Ark.*
- ROGERS, JAMES B., A.M., M.D., Associate Professor of Anatomy, *University of Louisville, School of Medicine, Louisville, Ky.*
- ROGERS, PHILIP V., Ph.D., Instructor in Biology, *Hamilton College, Clinton, N. Y.*
- ROGERS, WILLIAM MITCHELL, B.S., Ph.D., Assistant Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*
- ROMER, ALFRED S., B.A., Ph.D., Professor of Zoölogy and Curator of Vertebrate Palaeontology, *Biological Laboratories, Harvard University, Cambridge, Mass.*
- RONCORONI, ENRIQUE J., M.D., Professor of Anatomy, *Universidad del Litoral, Rosario, Republic of Argentina.*
- RONSTROM, G. N., M.D., Assistant Professor of Anatomy, *Louisiana State University Medical Center, 1542 Tulane Ave., New Orleans, La.*
- ROOPE, PAUL GIBBONS, B.S., Ph.D., Instructor in Anatomy, Department of Anatomy, *University of Louisville, School of Medicine, Louisville, Ky.*
- ROSSMAN, ISADORE, A.B., Ph.D., Research Assistant in Anatomy, *The University of Chicago, Chicago, Ill.*
- RUBINSTEIN, HYMAN S., M.D., Ph.D., Consultant in Psychiatry, U. S. Public Health Service; Director Research Laboratory, Surgical Division, Sinai Hospital, *2349 Eutaw Pl., Baltimore, Md.*
- RUDNICK, DOROTHEA, Ph.B., Ph.D., Instructor, *Albertus Magnus College, 700 Prospect St., New Haven, Conn.*
- RUGH, ROBERTS, B.A., M.A., Ph.D., Associate Professor of Zoology, New York University, *110 Morningside Drive, New York City.*
- RUTH, EDWARD S., M.D., F.A.C.S., *1680 North Vine St., Hollywood, Calif.*
- RUTH, ELBERT B., A.B., A.M., Ph.D., Assistant Professor of Anatomy, *College of Medicine, Eden Ave., University of Cincinnati, Cincinnati, O.*
- SABIN, FLORENCE R. B.S., M.D., Sc.D., LL.D. (Second Vice-Pres. '08-'09, Pres. '24-'26), Member Emeritus of Rockefeller Institute for Medical Research, *66th St. and York Ave., New York City; 1333 East 10th Ave., Denver, Colo.*
- SALSBUURY, C. R., M.D., C.M., F.R.C.S.(C.), Assistant Professor of Anatomy and Curator of Surgical Pathology, *Queen's University, Kingston, Ontario, Canada.*
- SANDSTROM, CARL J., Ph.D., Associate Professor of Biology, *New York University, New York City.*
- SAWIN, PAUL B., Sc.D., Assistant Professor of Biology, *Arnold Laboratory, Brown University, Providence, R. I.*

- SCAMMON, RICHARD E., Ph.D., LL.D. (Ex. Com. '23-'26, '35-'39, Vice-Pres. '28-'30), Distinguished Service Professor in the Graduate School, *University of Minnesota, Minneapolis, Minn.*
- SCHAEFFER, JACOB PARSONS, A.M., M.D., Ph.D., Sc.D. (Ex. Com. '24-'29), Professor of Anatomy and Director of the Daniel Baugh Institute of Anatomy, *Jefferson Medical College, 4634 Spruce St., Philadelphia, Pa.*
- SCHARRER, ERNST, M.D., Ph.D., Assistant Professor of Anatomy, *School of Medicine, Western Reserve University, 2109 Adelbert Rd., Cleveland, O.*
- SCHMIDT, IDA GENTHER, B.A., M.A., Ph.D., Assistant Professor of Anatomy, *University of Cincinnati, College of Medicine, Eden Ave., Cincinnati, O.*
- SCHOEMAKER, DANIEL M., B.S., M.D., Professor of Anatomy, *School of Medicine, St. Louis University, 1402 S. Grand Ave., St. Louis, Mo.*
- SCHOOLEY, J. P., Ph.D., Director, Endocrine Laboratories, *Difco Laboratories, Inc., Detroit, Mich.*
- SCHOUR, ISAAC, B.S., D.D.S., M.S., Ph.D., Professor of Histology and Head of the Department, *University of Illinois College of Dentistry, 808 South Wood St., Chicago, Ill.*
- SCHULTZ, ADOLPH H., Ph.D., Associate Professor of Physical Anthropology, Department of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- SCHWARTZ, HENRY G., M.D., Instructor in Neurological Surgery, *Washington University Medical School, Barnes Hospital, St. Louis, Mo.*
- SCHWEIZER, MALVINA, Ph.D., Instructor in Biology, *Washington Square College, New York University, 50 West 67th St., New York City.*
- SCHWIND, JOSEPH L., A.B., A.M., Ph.D., Associate Professor of Anatomy, *Albany Medical College, Albany, N. Y.*
- SCOTT, GORDON H., Ph.D., Associate Professor of Cytology, *Washington University School of Medicine, St. Louis, Mo.*
- SCOTT, JOHN W., A.M., Ph.D., Professor of Zoology and Research Parasitologist, *University of Wyoming, Laramie, Wyo.*
- SEDWICK, H. JOBE, D.D.S., *Algrunix Building, Titusville, Pa.*
- SEIB, GEORGE A., A.B., M.D., Assistant Professor of Anatomy, *Washington University, 2323 Lafayette Ave., St. Louis, Mo.*
- SELLING, LAURENCE, A.B., M.D., Professor of Medicine, *University of Oregon Medical School, 2228 S. W. 21st Ave., Mayer Building, Portland, Ore.*
- SELYE, HANS, M.D., Ph.D., Assistant Professor of Histology, *Department of Anatomy, McGill University, Montreal, Canada.*
- SEVERINGHAUS, AURA E., B.S., M.A., Ph.D., Associate Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, New York City.*
- SHANER, RALPH FAUST, Ph.D., Professor of Anatomy, *University of Alberta, Edmonton, Alberta, Canada.*
- SHANKLIN, WILLIAM M., Ph.D., Professor of Histology and Neuroanatomy, *American University of Beirut, Beirut, Syria.*
- SHAPIRO, HARRY H., D.M.D., Assistant Professor of Anatomy, *Columbia University, 630 West 168th St., New York City.*
- SHARP, CLAYTON, M.D., Assistant Professor of Anatomy, *Long Island College of Medicine, 350 Henry St., Brooklyn, N. Y.*

- SHEEHAN, DONAL, M.D., D.Sc., M.R.C.S., Professor of Anatomy and Director of the Anatomical Laboratories, *New York University, College of Medicine, 477 First Ave., New York City.*
- SHEININ, JOHN J., B.S., M.S., Ph.D., M.B., Dean and Chairman Department of Anatomy, *Chicago Medical School, 710 South Wolcott Ave., Chicago, Ill.*
- SHIELDS, RANDOLPH TUCKER, A.B., M.D., Associate Dean and Professor of Histology and Embryology, School of Medicine, *Shantung Christian University, Tsinan, Shantung, China.*
- SHUMWAY, WALDO, A.B., A.M., Ph.D., Professor of Zoölogy, *University of Illinois, Urbana, Ill.*
- SIMER, PARKE H., Ph.D., Assistant Professor of Anatomy, *University of Illinois, 1853 W. Polk St., Chicago, Ill.*
- SIMKINS, CLEVELAND S., A.B., Ph.D., Head, Department of Anatomy, *Creighton Medical School, Omaha, Neb.*
- SIMPSON, MIRIAM ELIZABETH, A.B., M.A., Ph.D., M.D., Associate Professor of Anatomy, *University of California, Berkeley, Calif.*
- SINGER, EDWARD, M.D., Associate Director, St. Clare's Hospital, *133 East 58th St., New York City.*
- SMELSER, GEORGE K., Ph.D., Assistant Professor of Anatomy, *Columbia University, College of Physicians and Surgeons, 630 West 168th St., New York City.*
- SMITH, BERTRAM GARNER, A.B., Ph.D., Professor of Anatomy, College of Medicine, *New York University, 477 First Ave., New York City.*
- SMITH, CARLTON G., B.A., M.Sc., M.D., Ph.D., Assistant Professor of Anatomy, *University of Toronto, Toronto 5, Canada.*
- SMITH, CHRISTIANNA, A.M., Ph.D., Professor of Zoölogy, *Mount Holyoke College, South Hadley, Mass.*
- SMITH, DAVID T., A.B., M.D., Associate Professor of Medicine and Professor of Bacteriology, *Duke University, Duke Hospital, Durham, N. C.*
- SMITH, GEORGE MILTON, A.B., M.D., Research Associate in Anatomy, *Yale University School of Medicine, Pine Orchard, Conn.*
- SMITH, PHILIP EDWARD, M.S., Ph.D. (Ex-Com. '34-'38), (Pres. '40-), Professor of Anatomy, College of Physicians and Surgeons, *Columbia University, 632 West 168th St., New York City.*
- SMITH, WILBUR CLEVELAND, B.A., M.D., Sc.D., Head of the Department of Gross Anatomy, *Tulane University, New Orleans, La.*
- SMITH, WILBUR K., A.B., M.D., Associate Professor of Anatomy, *University of Rochester School of Medicine and Dentistry, Rochester, N. Y.*
- SNIDER, RAY S., Ph.D., Research Fellow, *Johns Hopkins University, School of Medicine, Baltimore, Md.*
- SNODGRASS, L. E., A.B., M.D., Instructor in Surgery, Medical Department, *University of Pennsylvania, 7903 Oxford Ave., Philadelphia, Pa.*
- SNOOK, THEODORE, Ph.D., Assistant Professor of Anatomy, *Syracuse University, College of Medicine, Syracuse, N. Y.*
- SNYDER, FRANKLIN FAUST, M.D., Associate Professor, Department of Obstetrics, *Johns Hopkins Hospital, Baltimore, Md.*

- SOLNITZKY, OTHMAR, A.B., A.M., Ph.D., M.D., Professor and Director of the Department of Anatomy, Georgetown University, School of Medicine; Director, Georgetown University Brain Research Institute, *3900 Reservoir Rd., Washington, D. C.*
- SPAULDING, M. H., A.B., A.M., Professor of Zoölogy, Montana State College, *122 North Bozeman Ave., Bozeman, Mont.*
- SPECTOR, BENJAMIN, M.D., Professor of Anatomy, *Tufts College Medical School, 416 Huntington Ave., Boston, Mass.*
- SPEIDEL, CARL C., Ph.B., Ph.D. (Ex. Com. '40-), Professor of Anatomy, University of Virginia Medical School, *Charlottesville, Va.*
- STARK, MARY B., Ph.B., M.A., Ph.D., Sc.D., Professor of Histology and Embryology, *New York Medical College and Flower Hospital, Fifth Ave. and 105th St., New York City.*
- STEIN, KATHRYN F., M.S., Ph.D., Assistant Professor of Zoölogy, *Mount Holyoke College, South Hadley, Mass.*
- STEVENSON, PAUL HUSTON, B.S., M.A., M.D., Associate Professor of Anatomy, Peiping Union Medical College, Peiping, China, % *China Medical Board, 49 West 49th St., New York City.*
- STILES, HENRY WILSON, M.D., Professor of Anatomy, College of Medicine, Syracuse University, *R.D. no. 2, Mt. Kisco, N. Y.*
- STILWELL, E. FRANCES, A.B., A.M., Ph.D., Assistant Professor of Microscopic Anatomy, *Woman's Medical College of Pennsylvania, Philadelphia, Pa.*
- STONE, LEON STANSFIELD, Ph.B., Ph.D., Bronson Professor of Comparative Anatomy, *Yale University School of Medicine, 333 Cedar St., New Haven, Conn.*
- STONE, ROBERT S., B.A., M.A., M.B., M.D., Professor of Roentgenology, University of California Medical School, University Hospital, *Parnassus and Third Aves., San Francisco, Calif.*
- STOPFORD, JOHN SEBASTIAN B., M.D., Sc.D., D.Sc., F.R.S., Vice-Chancellor and Professor of Experimental Neurology, *University of Manchester, Manchester, England.*
- STRAUS, WILLIAM L., JR., Ph.D., Associate in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- STREETER, GEORGE L., A.M., M.D., Sc.D., LL.D., (Ex. Com. '18-'20, Pres. '26-'28), Research Associate, *Carnegie Institution of Washington, Johns Hopkins Medical School, 710 N. Washington St., Baltimore, Md.*
- STRÖER, W. F. II., Ph.D., Prosector in Anatomy, *University of Groningen, Groningen, Holland.*
- STROMSTEN, FRANK ALBERT, B.S., M.S., D.Sc., Associate Professor of Zoölogy, State University of Iowa, *318 Zoology Building, Iowa City, Ia.*
- STRONG, LEON H., A.B., B.S., Ph.D., Assistant Professor of Anatomy, Medical School, University of Michigan, *4502 E. Medical Building, University of Michigan, Ann Arbor, Mich.*
- STRONG, OLIVER S., A.B., A.M., Ph.D., Professor Emeritus of Neurology and Neurohistology, Columbia University, *Livingston Hall, 1116 Amsterdam Ave., New York City.*

- STRONG, REUBEN MYRON, A.B., A.M., Ph.D. (Ex. Com. '16-'19), Professor of Anatomy, *Loyola University School of Medicine, 706 South Wolcott Ave., Chicago, Ill.*
- STULTZ, WALTER A., B.A., Ph.D., Assistant Professor of Anatomy, *University of Vermont, College of Medicine, Burlington, Vt.*
- STUNKARD, H. W., Ph.D., Professor of Biology, *New York University, New York City.*
- SULLIVAN, WALTER EDWARD, A.M., Ph.D. (Ex. Com. '41-), Professor of Anatomy, *University of Wisconsin, Science Hall, Madison, Wis.*
- SUMMERS, FRANCIS M., Ph.D., Instructor in Biology, *College of the City of New York, 139th St. and Convent Ave., New York City.*
- SWANK, ROY L., B.S., M.B., M.D., Ph.D., Research Fellowship, *Department of Pathology, Harvard Medical School, Boston, Mass.*
- SWENSON, E. A., A.B., A.M., Ph.D., Associate in Anatomy, *Medical Department, University of Pennsylvania, Philadelphia, Pa.*
- SWETT, FRANCIS HUNTINGTON, A.B., A.M., Ph.D., Professor of Anatomy, *Duke University School of Medicine, Box 3710, Durham, N. C.*
- SWIFT, CHARLES H., M.D., Ph.D., Associate Professor of Anatomy, *The University of Chicago, Chicago, Ill.*
- SWINGLE, W. W., A.B., A.M., Ph.D., Professor of Biology, *Princeton University, Princeton, N. J.*
- SWINYARD, C. A., M.S., Ph.D., Associate Professor of Anatomy, *School of Medicine, University of Utah, Salt Lake City, Utah.*
- SYKES, GEORGE F., Ph.B., M.A., Assistant Professor of Anatomy, *Tufts College Medical School, 416 Huntington Ave, Boston, Mass.*
- TEITELBAUM, HARRY A., B.S., M.D., Ph.D., Assistant in Neurology, *Johns Hopkins University Medical School, 11 E. Chase St., Baltimore, Md.*
- TERNI, TULLIO, M.D., Professor of Anatomy, *The University of Padua, Padua, Italy.*
- TERRILL, CLAIRE ELMAN, B.S., Ph.D., Physiologist and Geneticist, *U. S. Western Sheep Breeding Laboratory, Dubois, Idaho.*
- TERRY, ROBERT-JAMES. A.B., M.D. (Ex. Com. '08-'12, '21-'24), Professor of Anatomy, *Washington University School of Medicine, St. Louis, Mo.*
- THARALDSEN, CONRAD E., B.S., M.A., Ph.D., Professor of Anatomy, *New York Medical College, 1 East 105th St., New York City.*
- THOMPSON, IAN MACLAREN, B.Sc., M.B., Ch.B., Professor of Anatomy and Director of the Department of Anatomy, *Medical College, University of Manitoba, Winnipeg, Canada.*
- THOMSON, STEWART CRAIG, A.B., M.D., M.S., Assistant Professor of Anatomy, *Loyola University School of Medicine, Chicago, Ill.*
- THORKEKELSON, JACOB, M.D., Member of Congress; MC-V (S) USNR, *Butte, Mont.*
- THURINGER, JOSEPH M., M.D., Professor of Histology and Embryology, *University of Oklahoma, School of Medicine, 801 N. E. 13th St., Oklahoma City, Okla.*
- TOBIN, CHARLES E., Ph.D., Instructor in Anatomy, *The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*
- TOMPKINS, EDNA H., M.D., Associate Professor of Anatomy, *Vanderbilt University Medical School, Nashville, Tenn.*

- TOWER, SARAH SHELDON, B.S., Ph.D., M.D., Associate in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- TRACY, HENRY C., A.B., A.M., Ph.D., Professor of Anatomy, *University of Kansas, Lawrence, Kan.*
- TROTTER, MILDRED, A.B., M.S., Ph.D., Associate Professor of Anatomy, *Washington University School of Medicine, St. Louis, Mo.*
- TRUEX, RAYMOND C., Ph.D., Instructor in Anatomy, *College of Physicians and Surgeons, Columbia University, New York City.*
- TURNER, CHARLES W., B.S., A.M., Ph.D., Professor of Dairy Husbandry, *University of Missouri, Columbia, Mo.*
- TURNER, C. DONNELL, Ph.D., Assistant Professor of Zoology, *Northwestern University, 105 Locy Hall, Evanston, Ill.*
- TURNER, CLARENCE L., M.A., Ph.D., Professor of Zoology, *Northwestern University, Evanston, Ill.*
- UHLENHUTH, EDUARD, Ph.D., Professor of Anatomy, *University of Maryland Medical School, Baltimore, Md.*
- UOTILA, UNTO U., M.D., Research Fellow in Anatomy, *Harvard Medical School, Boston, Mass. (1939)*; Docent in Microscopical Anatomy, *Helsinki University, Siltavuorenpenger 20, Helsinki, Finland.*
- VAN CAMPENHOUT, ERNEST, M.D., Ph.D., Professor of Anatomy, Histology and Embryology, *Université de Louvain, Louvain, Belgium.*
- VAN CLEAVE, C. D., Ph.D., Assistant Professor of Anatomy, *School of Medicine, University of North Carolina, Chapel Hill, N. C.*
- VAN DER HORST, C. J., D.Sc., Professor, Head of the Department of Zoölogy, *University of the Witwatersrand, Johannesburg, So. Africa.*
- VAN WAGENEN, GERTRUDE, A.B., Ph.D., Research Associate in Obstetrics and Gynecology, Associate Professor's rank, *Yale University, New Haven Hospital, New Haven, Conn.*
- VENABLE, JOHN HEINZ, B.S., M.D., Assistant Professor of Anatomy, *Emory University, Emory, Ga.*
- VICARI, EMILIA, M.A., Elizabeth Clay Howald Scholar, *Anatomy Department, College of Medicine, Ohio State University, Columbus, O.*
- VONDERAHE, A. R., A.B., B.Sc., M.D., Associate Professor of Anatomy, *University of Cincinnati, College of Medicine, Eden Ave., Cincinnati, O.*
- WAITE, FREDERICK CLAYTON, A.M., Ph.D., Emeritus Professor of Histology and Embryology, *Western Reserve University School of Medicine, 144 Locust St., Dover, N. H.*
- WALLER, WILLIAM H., Ph.D., Professor of Anatomy, *University of South Dakota, Medical School, Vermillion, S. Dak.*
- WALLIN, IVAN E., M.A., D.Sc., Professor of Anatomy and Head of Department, *Secretary of Faculty, University of Colorado, School of Medicine, Denver, Colo.*
- WALLS, G. L., Sc.D., Research Associate in Ophthalmology, *Ophthalmic Research Laboratory, Wayne University College of Medicine, 1512 St. Antoine St., Detroit, Mich.*
- WALMSLEY, THOMAS, M.D., F.R.S.E., Professor of Anatomy, *Queen's University of Belfast, Belfast, Ireland.*

- WARBRITTON, VIRGENE, M.A., Ph.D., (Mrs. Fred Kavanagh), *New York Botanical Gardens, Bronx, N. Y.*
- WARD, JAMES W., Ph.D., Instructor in Anatomy, *Vanderbilt University Medical School, Nashville, Tenn.*
- WARREN A. EMERSON, Ph.D., Associate Professor of Biology, *McMaster University, Hamilton, Ontario, Canada.*
- WARREN, CHARLES O., A.B., Ph.D., M.D., Research Associate in Anatomy, *Cornell Medical College, New York City.*
- WASSERMANN, FRIEDRICH, M.D., Associate Professor of Anatomy, *The University of Chicago, Chicago, Ill.*
- WATKINS, RICHARD WATKIN, S.B., M.D., Assistant Professor of Otolaryngology, *Rush Medical College, 5608 Kenwood Ave., Chicago, Ill.*
- WATT, JAMES CRAWFORD, M.A., M.D., Professor of Anatomy, *University of Toronto, 20 Hawthorne Ave., Toronto, Canada.*
- WEATHERFORD, HAROLD LORRAINE, A.B., A.M., Ph.D., Assistant Professor of Anatomy, *Harvard Medical School, Boston, Mass.*
- WEBB, RICHARD L., A.B., M.S., Ph.D., Associate Professor of Anatomy, *College of Medicine, University of Illinois, 1853 W. Polk St., Chicago, Ill.*
- WEED, LEWIS HILL, A.B., A.M., M.D., Sc.D., LL.D. (Ex. Com. '20-'21, '35-'39, Sec.-Treas. '22-'30), Professor of Anatomy and Director, *Johns Hopkins Medical School, Baltimore, Md.*
- WEICHERT, C. K., B.S., M.S., Ph.D., Associate Professor of Zoology, *University of Cincinnati, Cincinnati, O.*
- WEIDENREICH, FRANZ, Dr. Med., Honorary Director, *Cenozoic Laboratory, Peiping Union Medical College, Peking, China.*
- WEISS, PAUL, Ph.D., Associate Professor of Zoölogy, *The University of Chicago, Chicago, Ill.*
- WEISSENBERG, RICHARD, M.D., *School of Medicine, Middlesex University, Waltham, Mass.*
- WELLS, LEMEN J., A.M., Ph.D., Associate Professor of Anatomy, *Institute of Anatomy, University of Minnesota, Minneapolis, Minn.*
- WESTON, JEAN K., M.S., Ph.D., Assistant in Anatomy, *Temple University School of Medicine, Broad at Ontario St., Philadelphia, Pa.*
- WHEELER, THEODORA, A.B., M.D., Clinical Associate in Neurology, *Rush Medical College, 1758 W. Harrison St., Chicago, Ill.*
- WHITAKER, WAYNE L., A.B., A.M., Ph.D., Instructor in Anatomy, *University of Michigan, Ann Arbor, Mich.*
- WHITESIDE-HARVEL, BEATRICE, B.A., Ph.D., Voluntary Research Worker, *c/o Guaranty Trust Co., 140 Broadway, New York City.*
- WIEMAN, H. L., Ph.D., Professor of Zoology, *University of Cincinnati, Cincinnati, O.*
- WILLARD, WILLIAM A., A.M., Ph.D., Professor of Anatomy, *University of Nebraska, College of Medicine, Forty-second St. and Dewey Ave., Omaha, Neb.*
- WILLIAMS, GEORGE D., A.B., A.M., M.D., Ph.D., Assistant Professor of Anatomy, *Washington University School of Medicine, St. Louis, Mo.*
- WILLIAMS, ROY GRIFFIN, A.M., M.D., Associate Professor of Anatomy, *University of Pennsylvania School of Medicine, Philadelphia, Pa.*

- WILLIAMS, STEPHEN CULVER, Ph.D., Assistant Professor of Anatomy, *Medical Department, University of Pennsylvania, Philadelphia, Pa.*
- WILLIER, BENJAMIN HARRISON, Ph.D., Professor of Zoölogy, *University of Rochester, Rochester, N. Y.*
- WILSON, JAMES THOMAS, M.A., M.B., LL.D., F.R.S., Emeritus Professor of Anatomy, *University of Cambridge, 24 Millington Road, Cambridge, England.*
- WINDLE, WILLIAM F., B.S., M.S., Ph.D., Professor of Microscopic Anatomy, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*
- WISLOCKI, GEORGE B., A.B., M.D. (Ex. Com. '37-'41), Parkman Professor of Anatomy, *Harvard Medical School, Boston, Mass.*
- WITSCHI, EMIL, Ph.D., Professor of Zoology, *State University of Iowa, Iowa City, Ia.*
- WITTENBORG, A. H., M.D., Professor of Anatomy, *College of Medicine, University of Tennessee, Memphis, Tenn.*
- WOLFE, J. M., Ph.D., Associate Professor of Anatomy, *Albany Medical College, Albany, N. Y.*
- WOODBURNE, RUSSELL T., M.A., Ph.D., Assistant Professor of Anatomy, *University of Michigan, Ann Arbor, Mich.*
- WOODMAN, ALICE S., M.D., Associate Professor of Histology and Embryology, *Boston University School of Medicine, 80 East Concord St., Boston, Mass.*
- WORCESTER, JOHN LOCKE, M.D., Professor of Anatomy, *University of Washington, 5211 Twenty-first Ave., N. E., Seattle, Wash.*
- WRIGHT, WALTER H., D.D.S., B.S., M.S., Ph.D., Professor of Anatomy and Prosthetic Dentistry, *School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.*
- YNTEMA, C. L., Ph.D., Assistant Professor of Anatomy, *Cornell University Medical College, New York City.*
- YOFFEY, J. M., M.Sc., M.D., F.R.C.S., Professor of Anatomy, *The University, Bristol, Great Britain.*
- YOUNG, M. WHARTON, M.D., Ph.D., Assistant Professor of Anatomy, *Howard University, Washington, D. C.*
- YOUNG, WILLIAM C., Ph.D., Associate Professor of Primate Biology, *Yale Laboratories of Primate Biology, Orange Park, Fla.*
- YOUNGSTROM, KARL A., Ph.D., Instructor in Anatomy, *Duke University School of Medicine, Duke Station, Box 3608, Durham, N. C.*
- ZACHARIAS, LEONA RUTH, B.A., M.A., Ph.D., Instructor in Anatomy, *Columbia University, College of Physicians and Surgeons, New York City.*
- ZECHEL, GUSTAV, M.D., F.A.C.S., Assistant Professor in Anatomy, and Associate in Surgery, *University of Illinois College of Medicine, 1853 W. Polk St., Chicago, Ill.*
- ZEIT, WALTER, B.S., M.S., Ph.D., Assistant Professor of Anatomy, *Marquette University School of Medicine, Milwaukee, Wis.*
- ZIMMERMANN, ARNOLD, Lic.Sc., D.Sc. (Geneva, Switzerland), Professor of Anatomy, *University of Illinois College of Medicine, 1853 W. Polk St., Chicago, Ill.*
- ZWEIFACH, BENJAMIN WILLIAM, B.S., M.S., Ph.D. Research Associate in Biology, *New York University, New York City.*
- ZWEMER, RAYMUND L., A.B., Ph.D., Assistant Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*

